# EGFR RESISTANCE: PRIMARY AND SECONDARY: DIAGNOSIS, PROGNOSIS AND MANAGEMENT

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# INTRODUCTION

- Lung cancer is the leading cause of cancer- related mortality worldwide, with an **overall five-year survival rate of 15%**
- Non–small cell lung carcinoma **(NSCLC**) constitutes approximately **75-80% of all lung cancers**
- **~70% of patients** present with locally advanced or metastatic disease at the time of diagnosis **and are not eligible for surgical resection**
- **Platinum doublet therapy used to be the standard treatment option** in the past for these circumstances
- But these achieved a response rates of 30 to 40 % with a median survival of only **8 - 10 months**

**Santos et al.Annu. Rev. Pathol. Mech. Dis. 2011. 6:49–69**

# EGFR - BASICS

- EGFR gene is located on the short arm of chromosome 7 **(7p)**
- Encodes a 170-kDa transmembrane growth factor receptor with **tyrosine kinase activity**
- EGFR belongs to the **HER/*erb*B family of receptor** tyrosine kinases (RTKs), which includes HER1 (EGFR/*erb*B1), HER2 (*neu*, *erb*B2), HER3 (*erb*B3), and HER4 (*erb*B4)
- *EGFR*-mutant lung cancer was **first described** as a potential distinct clinical entity in **2004**

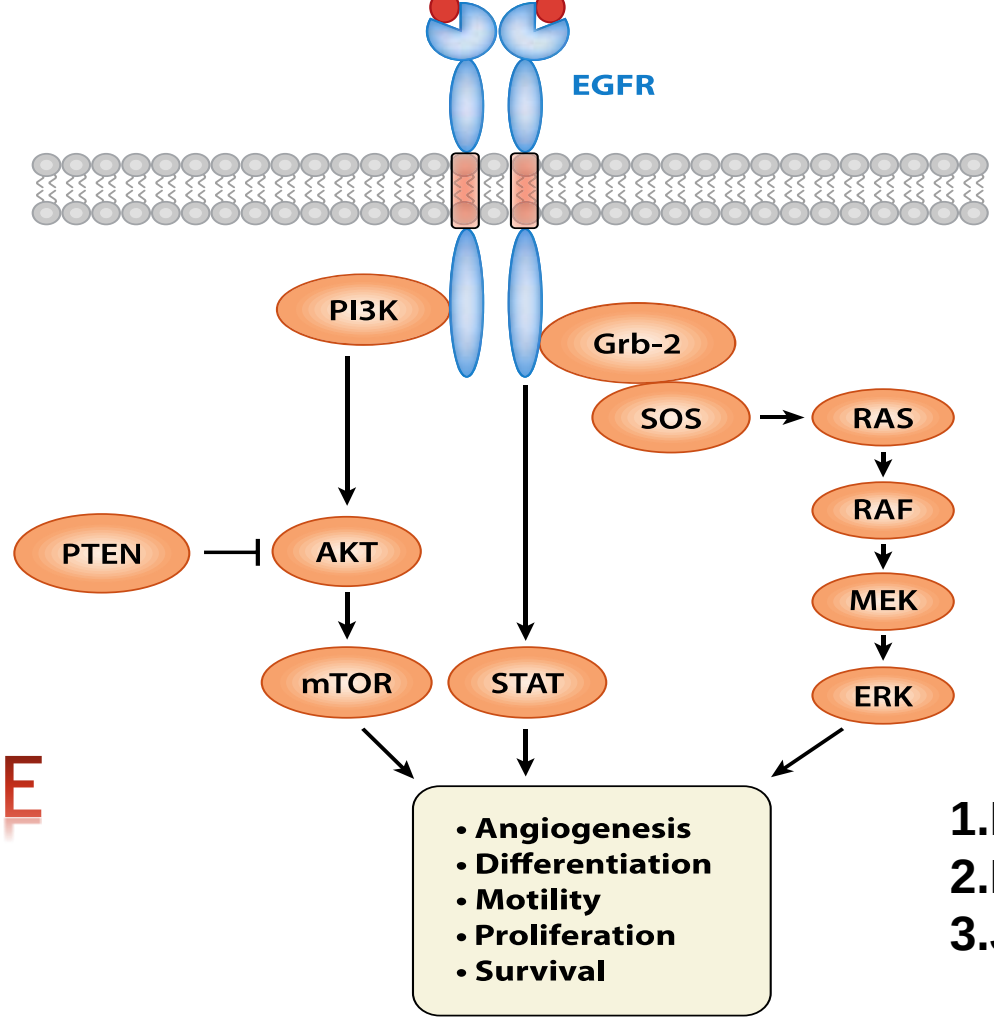
**Ohashi et al. *Journal of Clinical Oncology*. 2013;31(8):1070-1080**

# EGFR - BASICS STRUCTURE

- Extracellular, cysteine-rich ligand-binding domain
- A single α-helix transmembrane domain
- A cytoplasmic TK domain and a carboxy-terminal signalling domain
- Under normal circumstances, **binding of ligands (EGF, TGF-alpha) activates the intracellular tyrosine kinase** activity of EGFR via homo- or heterodimerization with EGFR family members
- In lung cancer, **EGFR mutations occur in exons encoding the ATP-binding pocket of the kinase domain (exons 18 to 21)**

**Ohashi et al. *Journal of Clinical Oncology*. 2013;31(8):1070-1080**

# EGFR STRUCTURE




**1.RAS/RAF/MAPK pathway**
**2.PI3K)/protein kinase B/ AKT pathway**
**3.JAK/ STAT)**

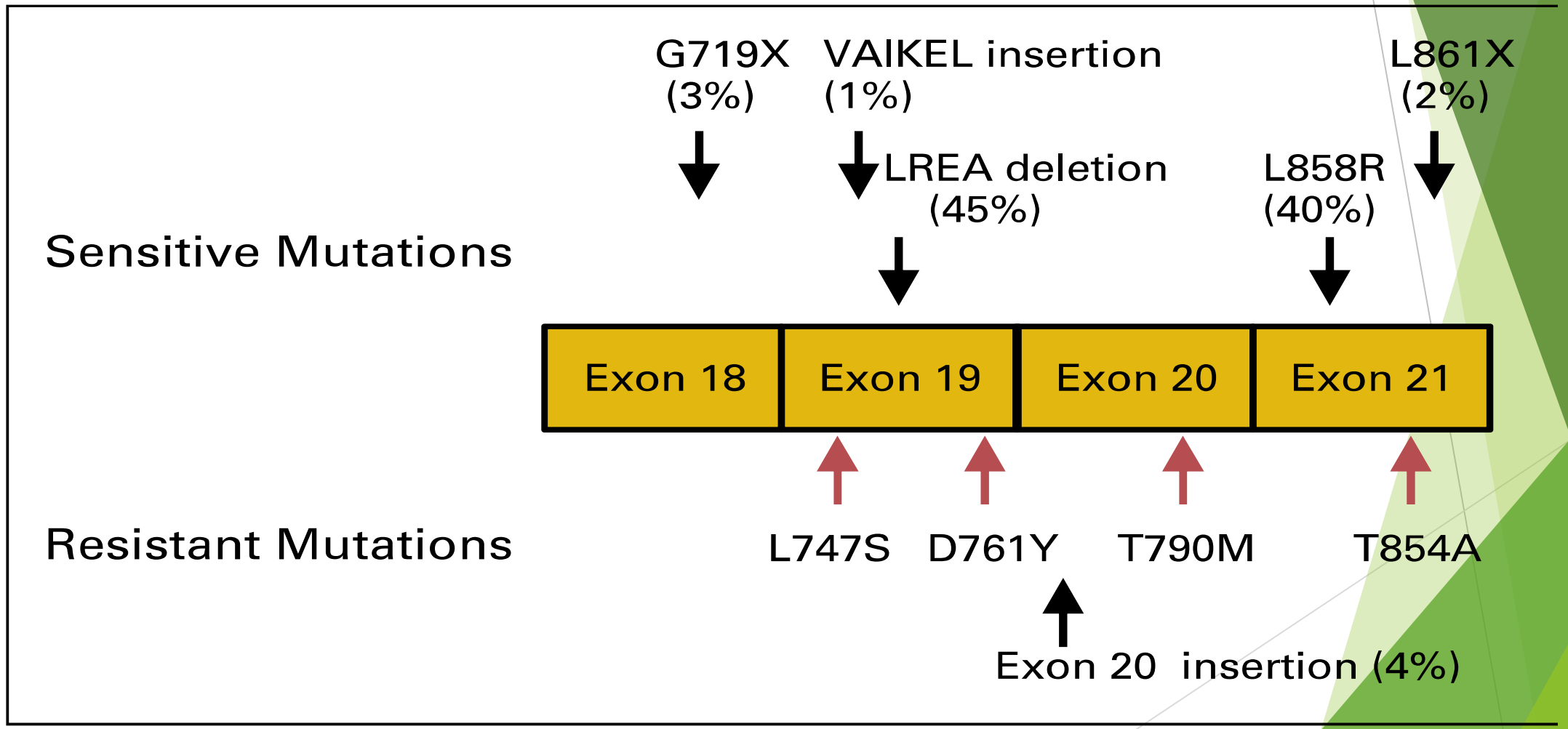
**Figure 1**

Simplified schema of epidermal growth factor receptor (EGFR)-induced signals that regulate critical cellular functions relevant to carcinogenesis. Abbreviations: ERK, extracytoplasmic-regulated kinase; Grb-2, growth factor receptor–bound protein 2; MAPK, mitogen-activated protein kinase; MEK, MAPK kinase; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; RAF, v-raf murine leukemia viral oncogene homolog; RAS, rat sarcoma viral oncogene homolog; SOS, sister of sevenless; STAT, signal transducer and activator of transcription.

**Santos et al. Annu. Rev. Pathol. Mech. Dis. 2011. 6:4**

**Huang et al . *Acta Pharmaceutica Sinica B*. 2015;5(5):390-401**

# EGFR - BASICS MUTATIONS




Ohashi et al. *Journal of Clinical Oncology*. 2013;31(8):1070-1080

# EGFR - BASICS MUTATIONS

- EGFR mutations lead to their constitutive and oncogenic activity
- Hence these mutations become a potential therapeutic target for lung cancer
- These mutations can be classified into
  a. **Drug sensitive and**
  b. **Drug resistant mutations**

**Ohashi et al. *Journal of Clinical Oncology*. 2013;31(8):1070-1080**

# EGFR SENSITIVE MUTATIONS

- **Deletions in exon 19** that eliminate a common amino acid motif (LREA) and point mutations in exon 21 that lead to a substitution of arginine for leucine at position 858 **(L858R)**
- **Exon 19 is the highest, accounting for more than 60% of overall mutations**
- Together, these two classes of mutations account for approximately **85% - 90% of EGFR mutations** in lung cancer- good sensitivity to TKIs
- Other less common mutations include **G719X (3%), L861X (2%),14 and exon 19 insertions (1%)- variable sensitivity to TKIs**

**Ohashi et al. *Journal of Clinical Oncology*. 2013;31(8):1070-1080**

# EGFR SENSITIVE MUTATIONS

- Found in **10% and 30%- 50%of unselected NSCLCs- North American/ European and East Asian countries respectively**
- Seen especially in those with **Adenocarcinoma** histology, history of **never smoking** cigarettes ( fewer than 100 cigarettes in a lifetime), **East Asian ethnicity, Female sex, bronchoalveolar carcinoma features in histopathology**, and papillary type (in some studies)

**Prevalence**

- East Asian non smokers with Adeno carcinoma- 60% to 80%
- North American/European non smokers with Adeno carcinoma- 30 to 50 %
- EGFR mutations (mostly exon 19 deletions and L858R point mutations) are associated with a clinical benefit from EGFR TKIs

**Ohashi et al. *Journal of Clinical Oncology*. 2013;31(8):1070-1080**

# EGFR INHIBITORS

1. MONOCLONAL ANTIBODIES:
   - Bind to the extracellular region of the receptor and function as competitive antagonists to inhibit ligand binding (eg Cetuximab)
2. SMALL MOLECULE TKIS :
   - Higher binding affinity for EGFR with sensitising mutation than do the wild-type receptors
   - **Overall response rates** among patients with *EGFR* mutant tumors **50% to 100%**
   - Response rates among patients with wild-type *EGFR* are 0% to 30%
   - Patients with EGFR mutations have a more favorable prognosis than do patients with wild-type *EGFR* irrespective of the treatment given

**Ohashi et al. *Journal of Clinical Oncology*. 2013;31(8):1070-1080**

**Santos et al. Annu. Rev. Pathol. Mech. Dis. 2011. 6:49-69**

| GENERATION | TKI | SELECTIVITY | REV/IRREVER | APPROVAL STATUS | FDA APPROVED DOSE/day | APPROVAL TIME |
|---|---|---|---|---|---|---|
| 1ST | Geftinib | WT EGFR | Reversible | FDA, EMA | 250 mg OD | As 1st line July 2015 |
| | Erlotinib | WT EGFR | Reversible | FDA, EMA | 150 mgOD | As 1st line May 2013 |
| | Icotinib | WT EGFR | Reversible | CFDA | 125 mg TDS | June 2011 |
| 2ND | Afatinib | WT EGFR + other HER | Irreversible | FDA, EMA, CFDA | 40 mg OD | As 1st line July 2013 |
| | Dacomitinib | WT EGFR + other HER | Irreversible | NO( awaiting) | - | - |
| 3RD | Osimertinib | MUTANT EGFR | Irreversible | FDA, EMA | 80 mg OD | As 2nd or 3rd line Nov 2015 **AS 1ST LINE APRIL 2018** |
| | Olmutinib | MUTANT EGFR | Irreversible | KFDA | 800mg/day | May 2016 |

**Ni J et al. *Chinese Medical Journal*. 2016;129(3):332-340**

**Westover et al. *Annals of Oncology*. January 2018;29(1): i10–i19**

**Kim et al. Drugs 2016 ;76(11): 1153**

| Trial | IPASS- 2009 ( PHASE 3 OPEN LABEL) | OPTIMAL - 2011 (PHASE 3 RCT) |
|---|---|---|
| SUBJECTS | **Previously untreated and advanced adenocarcinoma patients in East Asia** | Previously untreated EGFR mutation +ve advanced NSCLC with |
| METHOD | Geftinib 250 mg vs Carboplatin plus paclitaxel as first line therapy | Erlotinib 150 mg/day vs Gemcitabine plus carboplatin |
| RESULTS | EGFR mutation positive subjects (261): **Geftinib arm had higher PFS** (hazard ratio for progression or death, 0.48; 95% CI, 0.36 to 0.64; P<0.001 )<br>EGFR mutation negative subjects:<br>**PFS higher for chemo arm**<br>hazard ratio for progression or death with gefitinib, 2.85; 95% CI, 2.05 to 3.98; P<0.001 | RR 82 vs 36<br>CR 2 vs 0<br>PFS 13.1 months vs 4.6 months<br>OS 22.7 months vs 28.9 months |
| COMMENT | **Geftinib preferred as first line therapy for advanced adenoca lung with sensitive EGFR mutation** | Erlotinib preferred as first line therapy for advanced adenoca lung with sensitive EGFR |

**Ohashi et al. *Journal of Clinical Oncology*. 2013;31(8):1070-1080**

# TRIALS ON 2nd GEN EGFR TKIs

| STUDY | RCT - LUX LUNG 3 | RCT - LUX LUNG 6 |
|---|---|---|
| SUBJECT | 345 all EGFR mutant +ve | 364 all mutant +ve |
| METHOD | Afatinib vs Cisplatin/ pemetrexed | Afatinib vs Cisplatin/ Gem |
| RESULTS | PFS ( months ) 11.1 vs 6.9 | PFS ( months) 11 vs 5.6 |
| COMMENT | **Afatinib approved as 1st line for advanced EGFR +ve NSCLC** | |

# 1ST LINE TKI vs 2ND LINE TKI

| STUDY | LUX LUNG 7( MULTICENTRE RCT) | ARCHER 1050 ( MULTICENTRE RCT) |
|---|---|---|
| SUBJECTS | Stage IIIB /IV EGFR mutant +ve n= 319 | Stage IIIB/IV EGFR mutant +ve |
| METHOD | Afatinib vs Geftinib as 1st line | Dacomitinib 45 mg vs Geftinib 250 mg as 1st line |
| RESULT | **PFS** 11·0 months [95% CI 10·6–12·9] *vs* 10·9 months [9·1–11·5] Hazard ratio [HR] 0·73 [95% CI 0·57–0·95], p=0·017)<br>**TTF** 13·7 months [95% CI 11·9–15·0] *vs* 11·5 months [10·1–13·1]<br>**OS** 27.9 months vs 24.5 months<br>**ADVERSE EVENTS ( grade 3 and 4)-**<br>Rash or acne 9% vs 3%<br>Diarrhoea 13% vs 1%<br>Liver enzyme elevations- 0 vs 9%<br>Fatal – 9% vs 6% | **PFS** 14·7 months (95% CI 11·1-16·6) vs 9·2 months (9·1-11·0)<br>**OS** (months) 34.1 vs 26.8<br>**Grade 3-4 adverse events**<br>**Dermatitis acneiform** 14 % vs none<br>**Diarrhoea** 8% vs 1%, and<br>**Raised ALT** 1% vs 8%<br>**Serious adverse events** 9% vs 4% |
| COMMENT | 2nd generation Afatinib has better PFS than 1st gen Geftinib at a **higher incidence adverse events** | 2nd gen Dacomitinib better PFS and OS than Geftinib as 1st line but at a **higher incidence of adverse events** |

# WHY 2ND GENERATION BETTER -RESISTANCE

# EGFR RESISTANCE

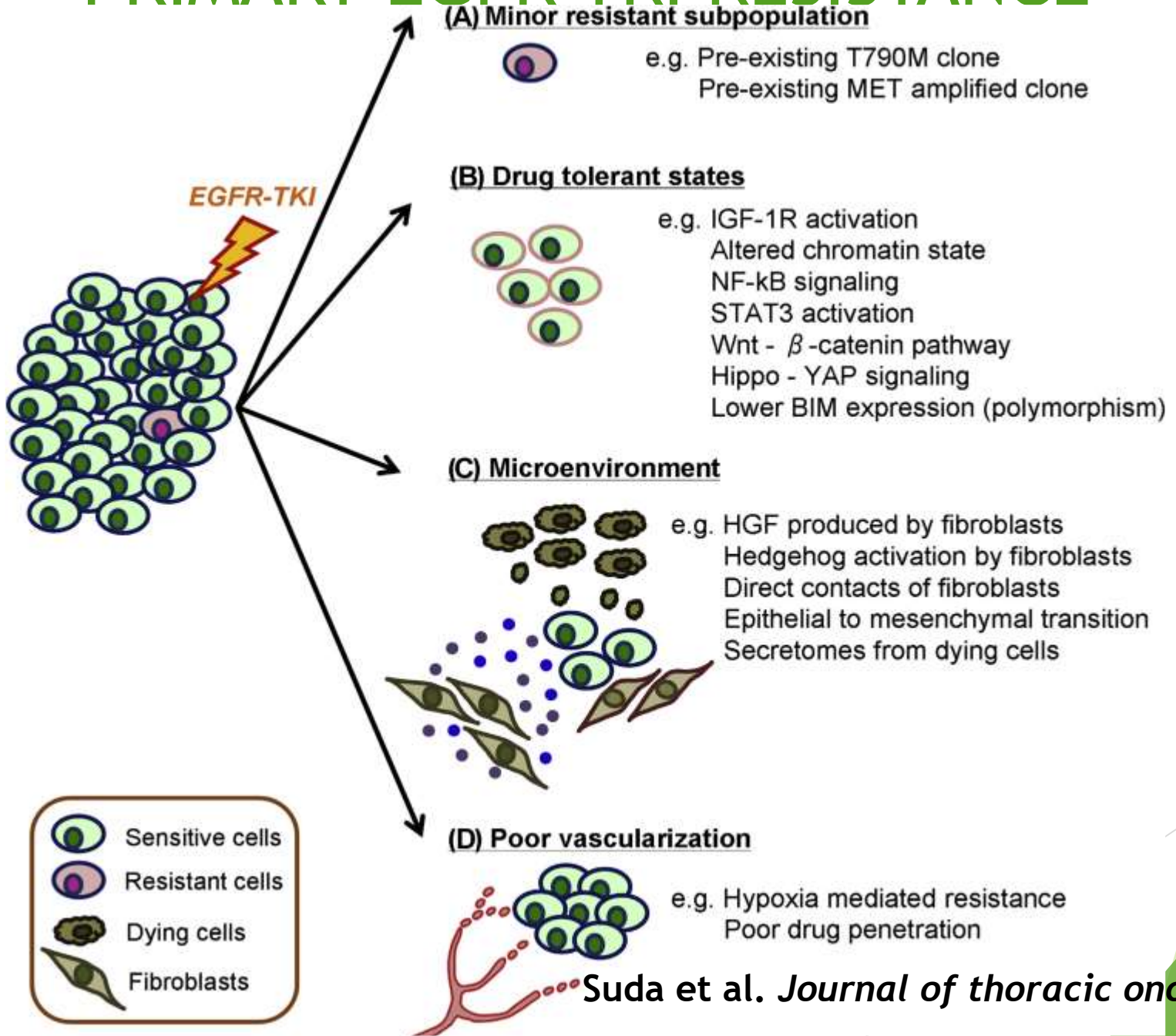
- Approximately **30% of patients still do not experience disease responses to EGFR TKIs despite harboring EGFR-mutant disease and less than 5% experience a complete response**
- Almost all the patients who receive EGFR TKIs progress after 9 to 13 months
- This is because of EGFR resistance which can be either :
- ✓ **Primary Resistance**: which occurs even before drug exposure to TKIs
- ✓ **Secondary Resistance :** which occurs following drug exposure to TKIs


**Huang et al. *Acta Pharmaceutica Sinica B*. 2015;5(5):390-401**

**Suda et al. *Journal of thoracic oncology*. 2017;12(1):27-35**

**Ohashi et al. *Journal of Clinical Oncology*. 2013;31(8):1070-1080**

# PRIMARY EGFR TKI RESISTANCE




Suda et al. *Journal of thoracic oncology*. 2017;12(1):27-35

# PRIMARY EGFR RESISTANCE

- 1. PREEXISTING MINOR SUBPOPULATION:
- Pre-existing T790M clone ( somatic/germline)

  (most common cause of primary resistance)
- Pre-existing MET amplified clone ( 2nd most common cause )
- Double T790M mutation plus MET amplification

Suda et al. *Journal of thoracic oncology*. 2017;12(1):27-35

# PRIMARY EGFR RESISTANCE

- Cancer cells may harbor several minor subpopulations with different EGFR mutations, including a TKI-resistant T790M mutation
- Pre-existent T790M has been reported **in 2.7%-40% of TKI-naïve patients**
- Prexistant minor T790M mutation/cMET amplification might cause decreased efficacy of EGFR TKIs
- These minor subpopulation of cells with prior resistant mutations are selected after starting TKIs because of their survival advantage

**Fang et al. Drug Des Devel Ther. 2014 Sep 26;8:1595-611.**

**Suda et al. *Journal of thoracic oncology*. 2017;12(1):27-35**

# PRIMARY EGFR RESISTANCE

| STUDY | JME ( prospective multicenter epidemiological study) |
|---|---|
| SUBJECTS | NSCLC STAGE I TO IIIB |
| METHOD | Using ddPCR to detect baseline T790M mutation status |
| RESULTS | Using analytical sensitivity of **0.001 %,** the overall incidence of the pretreatment T790M mutation was **79.9%** (298/373) and the frequency ranged from **0.009% to 26.9%.** The T790M mutation was detected more frequently in patients with a **larger tumor size (*P* = 0.019)** and those with **common EGFR-activating mutations (*P* = 0.022)**, as compared with the others. |

**T790M BEFORE TKI THERAPY**

- Allele frequency of the EGFR T790M mutation was between **0.001% and 0.1% in most of the cases (95%)**
- Cases **with abundant T790M allele (≥10%) were very rare (0.5%)**

**Suda et al. *Journal of thoracic oncology*. 2017;12(1):27-35**

# PRIMARY EGFR RESISTANCE

- Mediate **both primary and acquired resistance** to EGFR TKIs
- Reported to occur in **upto 21 % cases of TKI naïve NSCLC patients**
- Transmembrane Receptor tyrosine kinase
- Hepatocyte growth factor (HGF) triggers receptor dimerization and phosphorylation and activates downstream signalling pathway independent of EGFR kinase activity
- **HGF ligand overexpression**( another resistance mechanism) acts via cMET receptor

cMET AMPLIFICATION CLONES

**Faller et al. J Thorac Oncol. 2008 Apr;3(4):331-9**

**Huang et al. *Acta Pharmaceutica Sinica B*. 2015;5(5):390-401**

# PRIMARY EGFR RESISTANCE

2. REVERSIBLE DRUG-TOLERANT STATE :

- A small proportion of cells with EGFR sensitizing mutations remain **quiescent and uneliminated even after EGFR TKI therapy**
- This resistance mechanism is **reversible** and these cells will respond to EGFR monotherapy if exposed after a drug free period.
- However over a **longer period of time these quiescent cells acquire irreversible mutations** like T790M and MET amplification and show disease progression

**Suda et al. *Journal of thoracic oncology*. 2017;12(1):27-35**

# BIM POLYMORPHISM

- BIM (BCL2L11) is a BH3-only **proapoptotic member** of the Bcl-2 protein family
- Upregulation is required for apoptosis induction by EGFR-TKI in EGFR-mutant forms of NSCLC
- **BIM deletion polymorphism** occurs naturally **in 12.9% of East Asian individuals**, impairing the generation of the proapoptotic isoform and therefore conferring an inherent drug-resistant phenotype
- These individuals exhibited significantly inferior responses to EGFR-TKI treatment than individuals lacking this polymorphism
- **Pretreatment RNA levels of BIM** can predict the capacity of EGFR TKIs to induce apoptosis

Suda et al. *Journal of thoracic oncology.* 2017;12(1):27-35

Takayuki et al. Cancer Res. April 15 2013;73(8):2428-2434

# PRIMARY EGFR RESISTANCE

- 3. **MICROENVIRONMENT :**

Cancer cells receive survival signalling from the microenvironment that may modify drug efficacy

a. **Hepatocyte Growth factor-** secreted from fibroblasts and surviving lung cancer cells
b. **Hedgehog signalling** from fibroblasts
c. **Chemokines** - such as fibroblast growth factor or interleukin-8 from cancer cells

**Suda et al. *Journal of thoracic oncology*. 2017;12(1):27-35**

# PRIMARY EGFR RESISTANCE

d. **EMT ( Epithelial to Mesenchymal Transformation** )-Induced by fibroblasts and smoking

e. **Secretomes**- all proteins that are released by tumour cells into ECF from dying cancer cells

Suda et al. *Journal of thoracic oncology*. 2017;12(1):27-35

# PRIMARY EGFR RESISTANCE

- 4. **POOR VASCULARISATION**
- Hypoxic environment, and tumor hypoxia is associated **with aggressive tumor phenotypes, treatment resistance, and poor clinical prognosis**
- **Poor drug delivery to cancer cells causing a lower EGFR TKI concentration**, leading to earlier development of resistance than with higher drug concentrations
- **5. COEXISTING K RAS MUTATION**

**Suda et al. *Journal of thoracic oncology*. 2017;12(1):27-35**

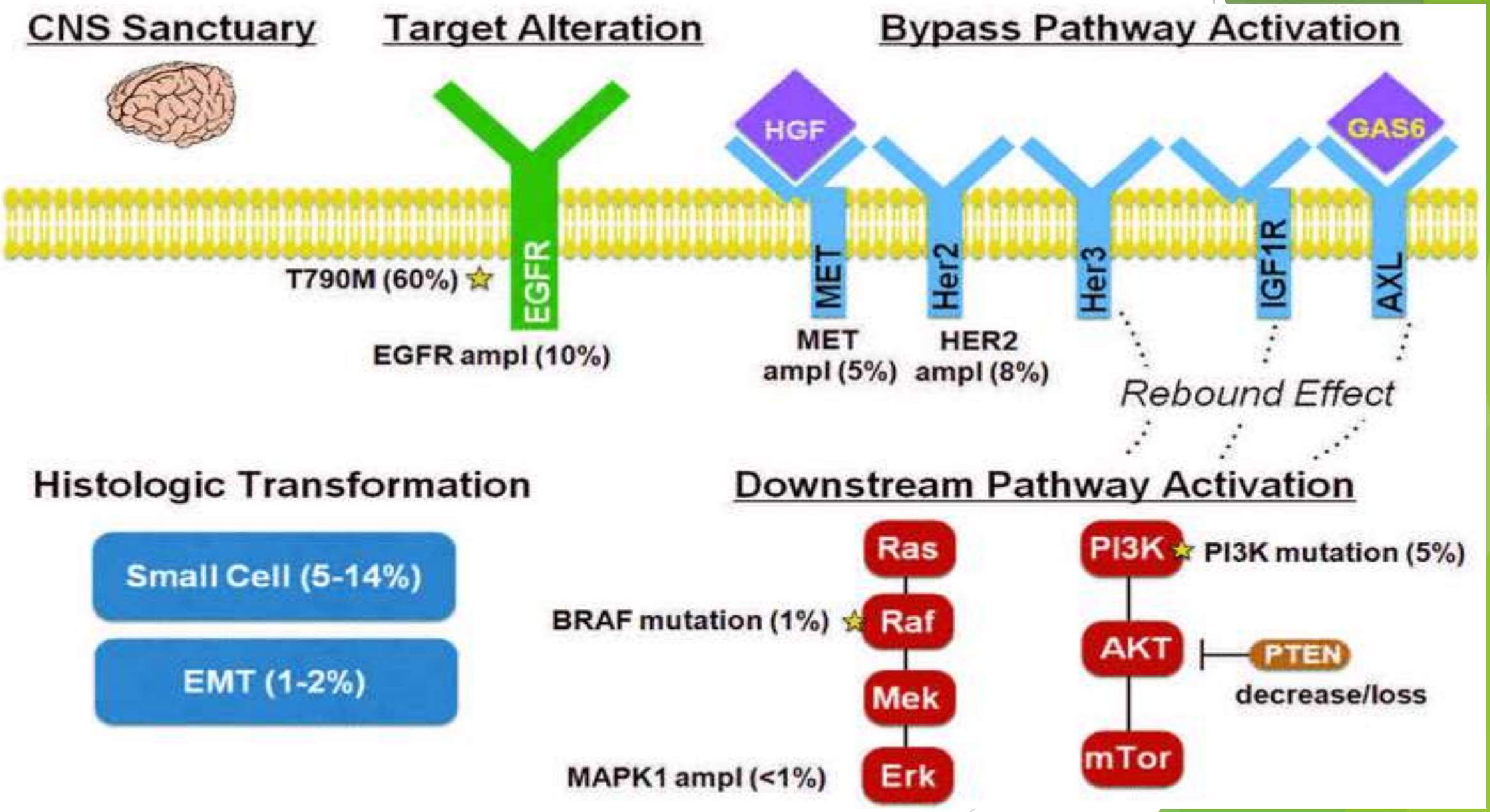
# K RAS MUTATION

| STUDY | METAANALYSIS | METAANALYSIS |
|---|---|---|
| SUBJECT | EGFR mutated NSCLC patients with and without K-RAS treated with TKIs 17 studies, 1008 patients, 165 had K RAS mutations | EGFR mutated NSCLC patients treated with EGFR TKIs 22 studies, consisting of 1470 NSCLC patients, of whom 231 had **KRAS mutations (16%)** |
| METHOD | Tested for complete Response and Partial Response stratified by K-RAS mutations | Patients were stratified according to their KRAS mutation status, gender, smoking history, histology, study treatment, CR and PR |
| RESULTS | **Presence of *k-RAS* mutations was significantly associated with an absence of response to TKIs (sensitivity=0·21 [95% CI 0·16-0·28], specificity=0·94 [0·89-0·97]; +LR=3·52; -LR=0·84)** | **K-RAS mutation frequency:**<br>**Smokers vs non-smokers -** 25% versus 6%; OR = 4.36; P < 0.01<br>**Adenocarcinoma vs other histologies** - 26% versus 16%; OR = 1.98; P < 0.01<br>**ORR of NSCLC patients with mutant KRAS vs WT KRAS** 3% vs 26%<br>The overall **pooled RR for ORR was 0.29** (95% CI: 0.18-0.47; P < 0.01). |
| COMMENT | **Highly specific negative predictor of response (de-novo resistance) to single-agent EGFR TKIs** | |

**C. Mao et al. / Lung Cancer *69 (2010) 272–278***

***Helena et al. Lancet Oncol* 2008; 9: 962–7**

# ACQUIRED EGFR RESISTANCE



# ACQUIRED EGFR RESISTANCE MECHANISMS

**1. SECONDARY EGFR MUTATIONS**

- **T790M MUTATION ( most common cause of acquired resistance to EGFR TKIs)**
  - Reported first in 2004
  - Present in 50%- 60% of the cases
  - Located in exon 20
  - Substitution of methionine for threonine at position 790 (T790M) in the kinase domain

**Huang et al. *Acta Pharmaceutica Sinica B*. 2015;5(5):390-401**

# ACQUIRED EGFR RESISTANCE MECHANISMS

- **T790M MUTATION**
- Enhances affinity of the ATP binding pocket for ATP, thus successfully **competing with the TKIs**, conferring resistance
- Can coexist with other mutations, like L858R and D761Y
- Enhanced phosphorylating activity, especially in combination with the L858R **mutation- T790M per se is an oncogene**

Huang et al. *Acta Pharmaceutica Sinica B*. 2015;5(5):390-401

# ACQUIRED EGFR RESISTANCE

- **Non T790M mutations ( PRIMARY/ ACQUIRED):**
- D761Y and L747S ( exon 19 ), T854A ( exon 21) and insertion mutation in exon 20
- **Modify the conformation of EGFR and the combination between EGFR and TKIs and inhibit BIM up-regulation**
- Frequency low
- **EGFR amplificatio**n – seen in 10% of patients who develop acquired resistance to TKI therapies and is always detected in the presence of EGFR T790M

**Huang et al. *Acta Pharmaceutica Sinica B*. 2015;5(5):390-401**

# ACQUIRED EGFR RESISTANCE

## 2. ABERRATED ACTIVATION OF THE BYPASS PATHWAYS

- Aberrance of other members of HER family ( HER2 overexpression)
- Amplification of c-Met **( Both primary and acquired )**
- Overexpression of HGF
- Abnormality of insulin growth factor receptor (IGFR) ( **both primary and acquired**)

**Huang et al. *Acta Pharmaceutica Sinica B*. 2015;5(5):390-401**

# ACQUIRED EGFR RESISTANCE

## 2. ABERRATED ACTIVATION OF THE BYPASS PATHWAYS

- The abnormality molecules of multiple angiogenic pathways **( both primary and acquired)**
  - The VEGFs and their receptors
  - The platelet-derived growth factors and their receptors (PDGFRs)

Huang et al. *Acta Pharmaceutica Sinica B*. 2015;5(5):390-401

# ACQUIRED EGFR RESISTANCE

- EGFRvIII
- Overexpression of or overactivated Anexelekto (AXL)
- Excess secretion of interleukin-6 (IL-6) – activates downstream JAK-STAT pathway
- Amplification of Crk-like protein (CRKL) -adapter protein that participates in signal transduction
- Overexpression and activation of integrin beta1-adhesion molecule

**Huang et al. *Acta Pharmaceutica Sinica B*. 2015;5(5):390-401**

# ACQUIRED EGFR RESISTANCE

3. ABNORMAL DOWNSTREAM PATHWAYS

- Loss of PTEN
- Mutations of BRAF
- Downstream mutation in PIK3CA
- Aberrant expression of NF1

Huang et al. *Acta Pharmaceutica Sinica B*. 2015;5(5):390-401

# ACQUIRED EGFR RESISTANCE

4. IMPAIRMENT OF EGFR TKI MEDIATED APOPTOSIS- BIM POLYMORPHISM **( primary and secondary)**

5.HISTOLOGIC TRANSFORMATION

- EMT
- Small cell transformation

6. ATP BINDING CASSETTE (ABC) EFFUSION

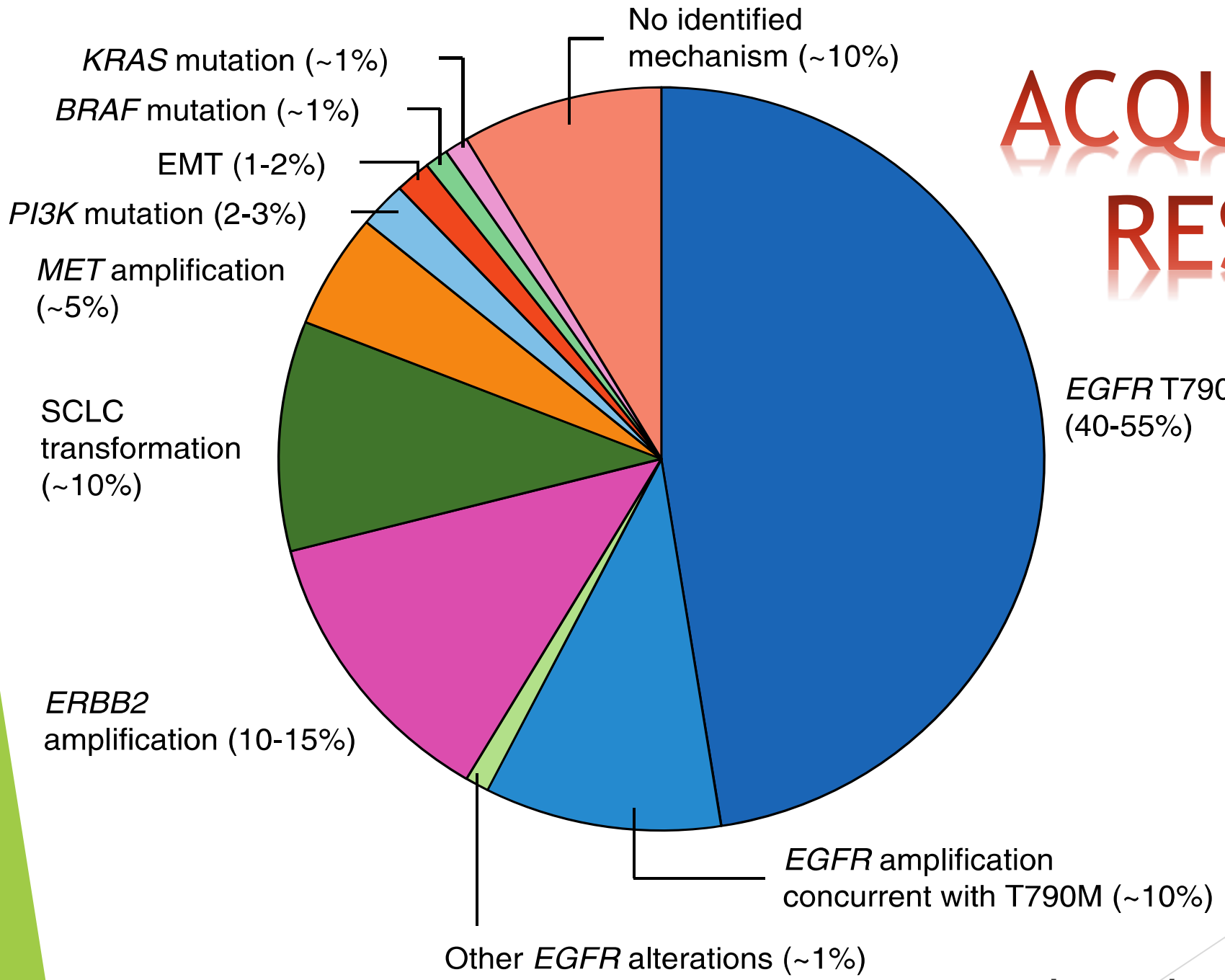
7. ALK SECONDARY MUTATION

8.CNS SANCTUARY

9.ALTERED DRUG PHARMACOKINETICS

**Huang et al. *Acta Pharmaceutica Sinica B*. 2015;5(5):390-401**

# ACQUIRED EGFR RESISTANCE




Westover et al. *Annals of Oncology.* January 2018;29(1): i10–i

# EMT TRANSFORMATION

- EGFR inhibition **induces TGFß secretion followed by SMAD pathway activation**
- Chronic exposure of *EGFR*-mutated NSCLC cells to TGFß sufficient to **induce EMT and hence resistance to EGFR TKI treatment**
- Characterized by a mesenchymal phenotype - combined **loss of epithelial cell junction proteins, such as E-cadherin, and the gain of mesenchymal markers, such as vimentin and N-cadherin**
- **Higher prevalence of the *EGFR* T790M mutated allele**
- **1-2 % cases after TKI therapy undergo EMT transformation**

**Soucheray et al. *Cancer Res*. 2015 October 15; 75(20): 4372-4383.**

# EGFRvIII

- EGFRvIII results from **in-frame deletion** of 801 base pairs spanning exons 2-7 of the coding sequence
- Removes 267 amino acids from the **extracellular domain, creating a junction site between exons 1 and 8 and a new glycine residue**
- Activation of **downstream PI3K/AKT/mTOR pathway and increases proliferation and cell cycle progression** mediated by a decrease in the level of p27
- Also has been shown to activate the NF-κB pathway, regulate IL-8 levels and angiogenesis

**Huang et al. *Acta Pharmaceutica Sinica B*. 2015;5(5):390-401**

# SMALL CELL TRANSFORMATION

- Less common phenomenon and only case reports ( incidences vary from 1 % to 26%)
- Conversion to small-cell carcinoma can occur at the time of development of resistance leading to **rapid worsening of the patient status**
- Retain the original EGFR mutation however EGFR expression is drastically reduced
- **Predictive Biomarkers:**
- A rapid increase in the serum NSE
- Pro-gastrin releasing-peptide (pro-GRP)

**Yangyang et al. Cancer Biology & Therapy. 2018;19(6):445-449**

# SMALL CELL TRANSFORMATION

- Proposed mechanisms-
- **Alveolar type II cells may be common precursors** of both lung adenocarcinoma and SCLC: might trans-differentiate to SCLC under the selective pressure of TKI therapy.
- Alternate hypothesis is that **initial tumours consisted of the combined histology of NSCLC and SCLC.** As the number of NSCLC cells decreased due to treatment, the SCLC component of the initial tumour became dominant
- SCLC tends to occur later in the earlier stage of adenocarcinoma (I, II, IIIA) than in advanced ones (IIIB, IV) – median time ( 59 months vs 20 months)

**Yangyang et al. Cancer Biology & Therapy. 2018;19(6):445-449**,

# ALK SECONDARY MUTATION

- Mostly EML4-ALK fusion gene occurs in those without RAS and EGFR mutations
- However **coexistence of both have been reported** and hence act as a bypass pathway for either given as monotherapy
- This can be overcome by combination treatment with both ALK and EGFR inhibitor

**Huang et al. *Acta Pharmaceutica Sinica B*. 2015;5(5):390-401**

# ALTERED DRUG PHARMACOKINETICS

- **Smoking**
- **Drugs**
  - Enzyme inducers
  - Usage of Antaacids/PPIs/H2 antagonists-TKIs are weak bases and concomitant intake of any of the above causes less acidic stomach and hence favor non—ionized form of the drugs and decrease the absorption of Geftinib and Erlotinib
  - Afatinib is unaffected by antaacids as it is insoluble over a wide pH range

**S. Peters et al. / Cancer Treatment Reviews 40 (2014) 91**

**Westover et al. *Annals of Oncology.* January 2018;29(1): i10–i19**

# ALTERED DRUG PHARMACOKINETICS

- **Food interaction**
  - Geftinib is unaffected by food
  - Erlotinib absorption is enhanced by food intake and should be taken 1 hour before or 2 hours after food intake
  - Afatinib is moderately affected by food itake and advised to be taken in the same manner as Erlotinib

**S. Peters et al. / Cancer Treatment Reviews 40 (2014) 91**

**Westover et al. *Annals of Oncology*. January 2018;29(1): i10–i19**

# DIAGNOSING ACQUIRED RESISTANCE Jackman Criteria-all should be met

1. Previously received treatment with a single agent EGFR TKI (eg Geftinib or Erlotinib)
2. Either of the following:
    1. A tumor that harbors an *EGFR* mutation known to be associated with drug sensitivity (ie, G719X, exon 19 deletion, L858R, L861Q)
    2. B. Objective clinical benefit from treatment with an EGFR TKI as defined by either:
        1. i. Documented partial or complete response (RECIST or WHO), or
        2. ii. Significant and durable ( 6 months) clinical benefit (stable disease as defined by RECIST or WHO) after initiation of gefitinib or erlotinib
3. Systemic progression of disease (RECIST or WHO) while on continuous treatment with gefitinib or erlotinib within the last 30 days
4. No intervening systemic therapy between cessation of gefitinib or erlotinib and initiation of new therapy

**Jackman et al. J Clin Oncol. 2010 Jan 10;28(2):357-60**

# DIAGNOSING MUTATION STATUS ( 2018 UPDATE)

## Guidelines From the College of American Pathologists, the IASCLC, and the Association for Molecular Pathology

- Stratified the bio-markers into 3 categories
- **"MUST-TEST" BIOMARKERS** - all patients with advanced lung cancer with an adenocarcinoma component who are being considered for an approved targeted therapy- **EGFR, ALK, ROS1**
- **"SHOULD-TEST" BIOMARKERS -** patients to clinical trials and which should be included in any large sequencing panel **- ERBB2, MET, BRAF, KRAS, and RET**
- **Remaining CANDIDATE BIOMARKERS ARE INVESTIGATIONAL** and are not appropriate for clinical use at this time.

**Lindeman et al. Journal of Thoracic Oncology Vol. 13 No. 3: 323-**

# DIAGNOSING MUTATION STATUS ( 2018 UPDATE) - SMEAR VS CELL BLOCK

| 2013 Statement | 2018 Statement |
| --- | --- |
| Expert consensus opinion: Cytologic samples are also suitable for EGFR and ALK testing, with cell blocks being preferred over smear preparations. | Recommendation: Pathologists may use either cell blocks or other cytologic preparations as suitable specimens for lung cancer biomarker molecular testing. |
| Expert consensus opinion: Laboratories should use EGFR test methods that are able to detect mutations in specimens with at least 50% cancer cell content, although laboratories are strongly encouraged to use (or have available at an external reference laboratory) more sensitive tests that are able to detect mutations in specimens with as little as 10% cancer cells. | Expert consensus opinion: Laboratories should use, or have available at an external reference laboratory, clinical lung cancer biomarker molecular testing assays that are able to detect molecular alterations in specimens with as little as 20% cancer cells. |
| Recommendation: Immunohistochemistry for total EGFR is not recommended for selection of EGFR TKI therapy. | Strong recommendation: Laboratories should not use total EGFR expression by IHC testing to select patients for EGFR-targeted TKI therapy. |

Lindeman et al. Journal of Thoracic Oncology Vol. 13 No. 3: 323-

# WHY IHC NOT SUITABLE FOR EGFR?

- EGFR mutations lead to activation of the cytoplasmic kinase of this trans- membrane protein, but that has no bearing on the extent of expression at the cell surface
- Also EGFR mutations detected using IHC show poor correlation to treatment response
- **Poor sensitivity for some exon 19 deletions, insensitivity to less common mutations** (eg, codon 719 mutations), and false-positive results with exon 20 insertions
- Overall, the performance is suboptimal for reliable detection of EGFR mutations

**Lindeman et al. Journal of Thoracic Oncology Vol. 13 No. 3: 323-**

# INDICATIONS FOR TESTING INITIAL MUTATION STATUS

- **All patients of lung adenocarcinoma** should be tested for EGFR, ALK and ROS 1 mutation status
- ERBB2, MET amplification status, RET amplification, BRAF, RAS mutation can be tested in lung adenocarcinoma as **a part of larger testing panel or when routine testing of EGFR/ALK/ROS 1 are negative**
- Molecular biomarker testing in tumors with histologies other adenocarcinoma can be done when clinical features indicate a higher probability of an oncogenic driver

**Lindeman et al. Journal of Thoracic Oncology Vol. 13 No. 3: 323-**

# TESTING MUTATION STATUS AFTER TUMOUR PROGRESSION

- EGFR mutation+ve adenocarcinoma, disease progression on 1st or 2nd line TKIs - Recommended to test for T790M mutation status
- Cell-free plasma DNA methods can be used to identify EGFR T790M mutations with progression or secondary clinical resistance to EGFR-targeted tyrosine kinase inhibitors - **testing of the tumor sample is recommended if the plasma result is negative**

*Not recommended to use ctDNA molecular analysis for the diagnosis of primary lung adenocarcinoma, the identification of EGFR or other mutations, or the identification of EGFR T790M mutations at the time of EGFR TKI resistance*

**Lindeman et al. Journal of Thoracic Oncology Vol. 13 No. 3: 323-**

# METHODS TO TEST

- ROS 1 – FISH is the gold standard ( RT PCR equally efficacious)
  - IHC has a sensitivity of 96 % specificity of 94% , hence can be used as a screening test before confirmation with FISH

- BRAF mutation – RT PCR or NGS testing ( INCLUDING TESTING FOR p.V600E)
- RET fusion rearrangements – FISH or RT-PCR or NGS testing
- ERBB2 mutation or amplification- NGS testing /FISH ( for amplification)
- RAS- NGS testing

**Lindeman et al. Journal of Thoracic Oncology Vol. 13 No. 3: 323-**

# METHODS TO TEST

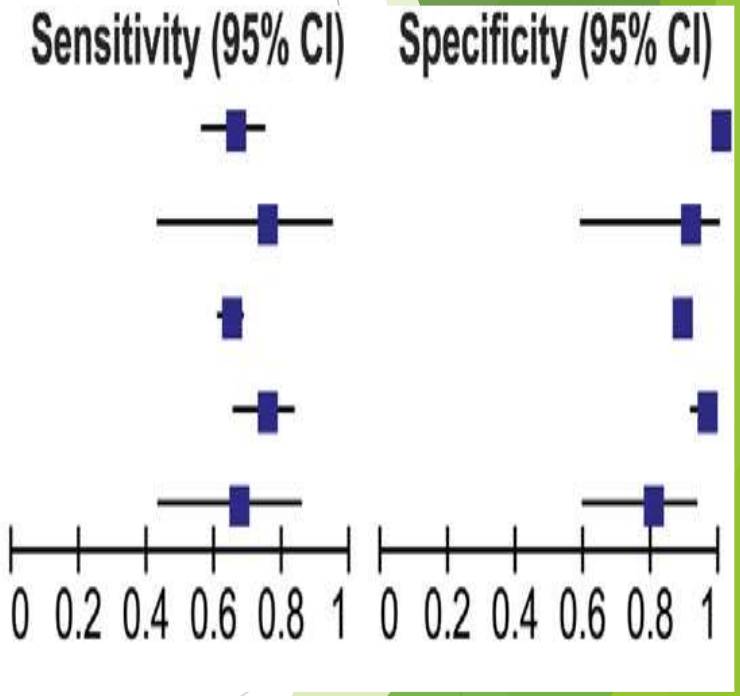
- MET AMPLIFICATION OR EXON 14 MUTATION-
- NGS testing or FISH ( for amplification)
- No guideline for cutoff of MET positivity in lung cancer specimens
- MET amplification has been classified by using MET:CEP7 ratio as low ( ≥1.8 to 2.2), intermediate (>2.2 to <5), and high (≥5)
- Only high MET amplifications have been considered to be oncogenic drivers
- Similarly only high MET amplifications have been shown to respond to MET inhibitors ( Capmatinib )

**Lindeman et al. Journal of Thoracic Oncology Vol. 13 No. 3: 323-**

# LIQUID BIOPSY

- Represent an integrative measure of all sites of disease
- Takes care of tumour heterogenicity

| Study | TP | FP | FN | TN | Detection System | Sensitivity (95% CI) | Specificity (95% CI) |
|---|---|---|---|---|---|---|---|
| Douillard 2014[242] | 69 | 1 | 36 | 546 | ARMs | 0.66 [0.56, 0.75] | 1.00 [0.99, 1.00] |
| Kukita 2013[234] | 9 | 1 | 3 | 10 | PNA/LNA clamp | 0.75 [0.43, 0.95] | 0.91 [0.59, 1.00] |
| Li 2014[243] | 389 | 114 | 214 | 874 | Multiple | 0.65 [0.61, 0.68] | 0.88 [0.86, 0.90] |
| Mok 2015[235] | 72 | 6 | 24 | 136 | allele-specific PCR | 0.75 [0.65, 0.83] | 0.96 [0.91, 0.98] |
| Oxnard 2014[232] | 14 | 5 | 7 | 20 | ddPCR | 0.67 [0.43, 0.85] | 0.80 [0.59, 0.93] |




Lindeman et al. Journal of Thoracic Oncology Vol. 13 No. 3: 323-

# PROGNOSTIC IMPLICATIONS OF EGFR MUTATION

- Several studies have been conducted on NSCLC patients stratified based on EGFR mutation status and their response to chemotherapy
- Most of these studies indicate that PFS and OS is better with EGFR mutation +ve patients without any significant difference in RR to chemotherapy
- It is postulated that **presence of EGFR mutation status is an independent prognostic marker and implies a favourable prognosis** for the patient because of better clinicopathologic characteristics

**Fang et al. Drug Des Devel Ther. 2014 Sep 26;8:1595-611.**

# T790M before starting TKI- Prognostic implication

| STUDY | METAANALYSIS |
|---|---|
| SUBJECT | **246 patients** with activating EGFR mutation such as Del19 or L858R participated in **4 selected trials** from **350 articles** |
| PURPOSE | Effect of pre treatment T790M mutation on the survival of patients with EGFR mutation treated with TKI |
| RESULT | Overall incidence of patients with pretreatment T790M mutation was **43.10%** (106/246), ranging from 34.88% to 80.00% in the individual trials<br>Combined hazard ratio for PFS in all four eligible studies was **2.602** (95% confidence interval 1.011-6.695; P=0.047) |
| COMMENT | Pre-treatment T790M mutation **had a negative impact on the PFS of non-small cell lung cancer patients with a Del19 or L858R EGFR mutation who received EGFR TKI treatment.** |

PRETREATMENT T790M-NEGATIVE PROGNOSTIC MARKER

Ding et al. *OncoTargets and therapy*. 2014;7:387-393

# T790M POST TKI- PROGNOSTIC IMPLICATION

| STUDY | PROSPECTIVE |
|---|---|
| SUBJECTS | EGFR TKI treated NSCLC EGFR mutation +ve patients who progressed with and without T790M mutation<br>N= 93 |
| METHOD | Effect of post progression T790M mutation on survival |
| RESULTS | T790M +ve (58/93)<br>Median post-progression survival was **16 months** (interquartile range 9–29 months) **19 months vs 12 months p - 0.036**<br>Patients with T790M were more **likely to progress in an existing site of disease rather than a new organ system**<br>Patients without T790M more often progressed in a previously uninvolved organ system **(p=0.014)** and exhibited a poorer performance status at time of progression **(p=0.007)**<br>**TTP 14 vs 11 months ( p-0.10)** |
| COMMENTS | Durable responses to EGFR TKIs<br>Distinct biology |

**Oxnard et al. Clin Cancer Res. 2011 Mar 15;17(6):1616-22**

# IN GENERAL…

The absence of T790M after progression, likely indicate some “other” resistance mechanism, and is associated with **earlier development of new metastatic sites of disease and a poorer performance status, contributing to the shorter survival of these patients**

**Oxnard et al. Clin Cancer Res. 2011 Mar 15;17(6):1616-22**

# TREATMENT

# WAYS OF TACKLING DENOVO T790M mutation

| STUDY | BELIEF ( multicenter single arm trial) | ACCRU (RCT) |
|---|---|---|
| SUBJECT | Treatment naive, IIIB or stage IV lung adenocarcinoma with activating EGFR mutation | stage IIIB/IV or recurrent non-squamous NSCLC with activating EGFR mutations |
| METHOD | oral **erlotinib 150 mg/day + intravenous bevacizumab** 15 mg/kg every 21 days and were tested for the pretreatment T790M resistance mutation | Erlotinib 150 mg/day plus bevacizumab 15 mg/kg every 3 weeks or erlotinib 150 mg/day monotherapy as a first-line therapy |
| RESULTS | **T790M-positive group**<br>**PFS-16·0 months** (12·7 to not estimable), with a 12 month progression-free survival of 68% (50-81)<br>**T790M-negative group**,<br>**PFS-10·5 months** (9·4-14·2), with a 12 month progression-free survival of 48% (36-59). | **PFS - 16·0 months** (95% CI 13·9-18·1) vs **9·7 months** (5·7-11·1) (hazard ratio 0·54, 95% CI 0·36-0·79; log-rank test p=0·0015)<br>**Adverse effects more in the combination arm** |
| COMMENT | EGFR TKI + VEGF inhibitors can be used as a **first line therapy in EGFR mutation positive status and it delays the onset of T790M mutation**<br>**Ramucirumab** is also undergoing clinical trials | |

**Seto et al. Lancet Oncol. 2014 Oct;15(11):1236-44.**
**Rosell et al. Lancet Respir Med. 2017 May;5(5):435-444**
**Westover et al. *Annals of Oncology*, Volume 29, Issue suppl_1, 1 January 2018, Pages i10–**

# TACKLING cMET AMPLIFICATION combining EGFR TKI with MET inhibitors

- Since MET amplification is the second most common mechanism of acquired resistance following EGFR TKI, combining both EGFR TKI and MET inhibitors is a rational approach
- Various studies have been conducted on combining both MET inhibitors and EGFR TKIs with varying results
- Studies using MET inhibitors include **crizotinib, tivantinib, cabozantinib, volitinib and onartuzumab** have been conducted following progression after EGFR TKIs
- Crizotinib has ALK,ROS1 and MET inhibitor property

**Suda et al. *Journal of thoracic oncology*. 2017;12(1):27-35**

# MET INHIBITOR PLUS EGFR TKI

| STUDY | Prospective | Prospective |
|---|---|---|
| SUBJECTS | Advanced NSCLC patients post chemotherapy ( IRRESPECTIVE OF EGFR/MET STATUS) | Advanced NSCLC patients post chemo( IRRESPECTIVE OF EGFR/MET STATUS) |
| METHOD | Safety and efficacy of EGFR TKI plus MET inhibitor<br>( Erlotinib plus crizotinib) , no control group | EGFR TKI + MET inhibitor ( Erlotinib + onartuzumab) vs (Erlotinib + placebo) |
| RESULTS | MTD was less than the approved dose for both due to adverse events | MET +ve patients (n = 66) treated with erlotinib + onartuzumab showed improvement in both PFS (HR 0.53; P = 0.04) and OS (HR, .37; P =0.002)<br>But worse outcomes if same used in MET negative patients |
| COMMENTS | Negative study and phase 2 not initiated as it was done on mutation unselected patients | Imbalance in EGFR mutation prevalence between the groups, hence no definite conclusion |

Ou, Sai-Hong Ignatius et al. Journal of Thoracic Oncology. 2017;12 (1):145-151
Suda et al. *Journal of thoracic oncology*. 2017;12(1):27-35
Spigel et al. *Journal of Clinical Oncology*. 2013;31(32):4105-4114.

# MET inhibitor + EGFR TKI

| STUDY | METLung TRIAL |
|---|---|
| SUBJECTS | Post Platinum doublet chemo advanced NSCLC progression with MET + status (n=499) |
| METHOD | (Erlotinib 150 mg+ onatuzumab 15 mg/kg) vs (Erlotinib 150 mg+ placebo) |
| RESULTS | OS 6.8 VS 9.1 months<br>HR 1.27<br>PFS 2.7 vs 2.6 months<br>ORR 8.4 % VS 9.6 % |
| COMMENT | No improvement in post chemo MET amplification+ve NSCLC patients using combined Erlotinib + Onartuzumab |

**Spigel et al. Journal of Clinical Oncology 2017 35:4, 41**

# Still…Hope Not lost…

- Despite the negative results from the prior mentioned studies, there are case reports of drastic improvement following combined EGFR TKI and MET inhibitors in patients with EGFR mutation and MET amplification primary mutations


CASE REPORT

## Dramatic Response to Combination Erlotinib and Crizotinib in a Patient with Advanced, *EGFR*-Mutant Lung Cancer Harboring De Novo *MET* Amplification

Justin F. Gainor, MD,[a,*] Matthew J. Niederst, PhD,[a] Jochen K. Lennerz, MD, PhD,[b] Ibiayi Dagogo-Jack, MD,[a] Sara Stevens, NP,[a] Alice T. Shaw, MD, PhD,[a] Lecia V. Sequist, MD, MPH,[a] Jeffrey A. Engelman, MD, PhD[a]

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## Response to crizotinib/erlotinib combination in a patient with a primary *EGFR* mutant adenocarcinoma and a primary *c-met* amplified adenocarcinoma of the lung

**Martin Frederik Dietrich, MD, PhD**[1], **Shirley Xiao Yan, MD**[2], and **Joan Hoff Schiller, MD**[1]

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[2]Department of Pathology, UT Southwestern Medical Center, 5300 Harry Hines Boulevard, Dallas, TX 75390

# Future prospective with MET…

- Recently MET exon 14 mutations have been found in NSCLC
- **MET exon 14 alterations** have been found to be driver mutations of their own and Crizotinib has been found to be effective in lung cancer patients with these mutations
- Hence future studies need to focus on using combining EGFR and MET inhibitors after progression following EGFR TKI monotherapy especially in those who have acquired resistance using MET amplification
- Also RCT using MET inhibitors in MET exon 14 mutations is warranted

Drilon et al. Journal of Thoracic Oncology , Volume 12 , Issue 1 , S438 - S439

Mark et al. Journal of Clinical Oncology 2016 34:7, 721-7

# IGF-1R INHIBITORS BEING STUDIED

**Table 1.** Monoclonal antibodies that target the type I insulin-like growth factor receptor (IGF-1R) pathway

| Target | Agent name | Sponsor | Status | Class | Phase 2 dose |
|---|---|---|---|---|---|
| IGF-1R | Cixutumumab (IMC-A12)[49,84] | ImClone | Phase 2 | IgG1 | 6 mg/kg qw, 10 mg/kg q2w |
| IGF-1R | Figitumumab (CP-751,871)[35,36,59] | Pfizer | Discontinued after Phase 3 | IgG2 | 20 mg/kg q3w |
| IGF-1R | Dalotuzumab (MK-0646; h7C10)[85,86] | Pierre Fabre and Merck | Phase 3 | IgG1 | 10 mg/kg q2w |
| IGF-1R | Ganitumab (AMG 479)[33] | Amgen | Phase 3 | IgG1 | 18 mg/kg q3w |
| IGF-1R | R1507[41] | Roche | Phase 2 | IgG1 | 9 mg/kg qw |
| IGF-1R | SCH 717454 (19D12)[37] | Schering Plough | Discontinued after Phase 1 | IgG1 | NA |
| IGF-1R | AVE1642 (EM164)[38] | ImmunoGen/Sanofi | Discontinued | IgG1 | 8 mg/kg q4w, 12 mg/kg q3w |
| IGF-1R | BIIB022[87,88] | Biogen-IDEC | Discontinued after Phase 1 | IgG4 | NA |
| IGF-1 and IGF-2 | MEDI-573[27,89] | MedImmune | Phase 1 | IgG2 | NA |

NSCLC

Chen et al. *Chinese Journal of Cancer*. 2013;32(5):242-252.

| STUDY | Prospective |
|---|---|
| SUBJECTS | Advanced-stage NSCLC with progression following one or two prior chemo regimens |
| METHOD | Erlotinib + placebo vs<br>Erlotinib + R1507 9 mg/kg weekly vs<br>Erlotinib + R1507 16 mg/kg i.v once every 3 weeks. |
| RESULTS | 12-week PFS - 39% vs 37% vs 44%<br>Median OS - 8.1 vs 8.1 vs 12.1 months<br>**But improved PFS in KRAS mutation +ve patients** |
| COMMENTS | Combination of R1507 with erlotinib **did not provide PFS or survival advantage over erlotinib alone** in an unselected group of patients with advanced NSCLC. |

NEED SPECIFIC STUDIES ON IGF-1R + AND KRAS NSCLC PTS

**R1507 is a selective, fully human, recombinant monoclonal antibody (immunoglobulin G1 subclass) against insulin-like growth factor-1 receptor (IGF-1R)**

Ramalingam et al. J Clin Oncol: 2011; 29:4574-4580

# NF-KB pathway-Any hope

Therapeutics, Targets, and Chemical Biology

Cancer Research

# Inhibition of Casein Kinase 1 Alpha Prevents Acquired Drug Resistance to Erlotinib in EGFR-Mutant Non–Small Cell Lung Cancer

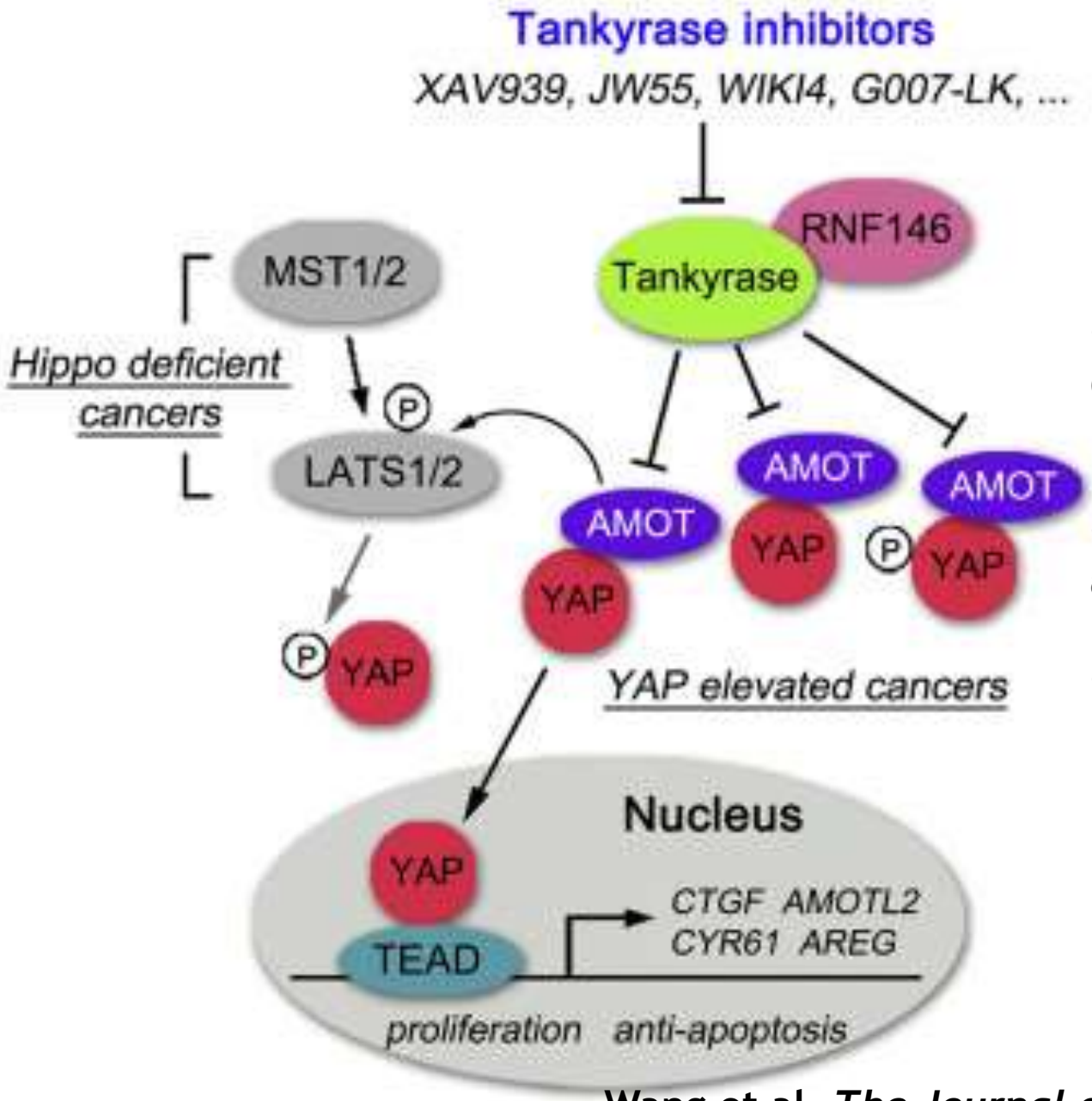
Alexandra B. Lantermann, Dongshu Chen, Kaitlin McCutcheon, Greg Hoffman, Elizabeth Frias, David Ruddy, Daniel Rakiec, Joshua Korn, Gregory McAllister, Frank Stegmeier, Matthew J. Meyer, and Sreenath V. Sharma

Lantermann et al. Cancer Res. 2015 Nov 15;75(22):4937-48

## Abstract

Patients with lung tumors harboring activating mutations in the EGF receptor (EGFR) show good initial treatment responses to the EGFR tyrosine kinase inhibitors (TKI) erlotinib or gefitinib. However, acquired resistance invariably develops. Applying a focused shRNA screening approach to identify genes whose knockdown can prevent and/or overcome acquired resistance to erlotinib in several EGFR-mutant non–small cell lung cancer (NSCLC) cell lines, we identified casein kinase 1 α (CSNK1A1, CK1α). We found that CK1α suppression inhibits the NF-κB prosurvival signaling pathway. Furthermore, downregulation of NF-κB signaling by approaches independent of CK1α knockdown can also attenuate acquired erlotinib resistance, supporting a role for activated NF-κB signaling in conferring acquired drug resistance. Importantly, CK1α suppression prevented erlotinib resistance in an HCC827 xenograft model *in vivo*. Our findings suggest that patients with EGFR-mutant NSCLC might benefit from a combination of EGFR TKIs and CK1α inhibition to prevent acquired drug resistance and to prolong disease-free survival.

*Cancer Res; 75(22); 4937–48.* 




- The Tankyrase axis promotes the degradation of angiomotin family proteins and provides a bypass for tumour cells
- Tankyrase inhibitors target YAP by stabilizing angiomotin family proteins
- This further prevents bypass pathway for EGFR resistance and increases sensitivity to EGFR TKIs

Wang et al. Cell Reports .2015:13; 524–

Wang et al. *The Journal of Biological Chemistry*. 2016;291(29):15256-15266

# Tankyrase Inhibitor- Is it possible?

| STUDY | PRECLINICAL EVALUATION |
|---|---|
| SUBJECTS | NSCLC CELL LINES |
| METHOD | Tankyrase inhibitor AZ1366 in combination with multiple EGFR-inhibitors across NSCLC lines, characterizing its anti-tumor activity, impingement on canonical Wnt signaling and effects on gene expression. |
| RESULTS | AZ1366 **synergistically suppressed proliferation of multiple NSCLC lines and amplified global transcriptional changes brought about by EGFR- inhibition** |
| COMMENTS | Bright prospective for future human trials using combined Tankyrase inhibitor and EGFR TKIs to prevent/delay resistance |

Scarborough et al. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2017;23(6):1531-1541.

# TACKLING PREEXISTING BIM POLYMORPHISM

- Histone deacetylase (HDAC) inhibitor vorinostat could circumvent EGFR-TKI resistance in EGFR-mutant NSCLC cell lines that also harbored the BIM polymorphism
- A preclinical trial was conducted on cell lines with EGFR mutation + BIM polymorphism
- In those with **BIM polymorphism EGFR TKI + Vorinostat = EGFR TKI alone in those without BMI polymorphism**
- **Mechanism : HDAC removes acetyl groups from histone and non-histone proteins and causes their stabilisation** thereby promoting cell proliferation
- **HDACi causes destabilisation of histone and non histone proteins thereby** causing cell cycle arrest and apoptosis

Takayuki et al. Cancer Res. April 15 2013;73(8):2428-2434

# ACQUIRED RESISTANCE

- Three clinical subtypes of acquired resistance according to the extent and sites of progressive disease are generally accepted:
- (i) systemic or multi-site progression ( 60 -70%)
- (ii) oligo- progression (three or less progressing locations) ( 20 - 25%), and
- (iii) isolated CNS progression ( 15%)

**Westover et al. *Annals of Oncology.* January 2018;29(1): i10–i19**

# ACQUIRED RESISTANCE

- For patients developing T790M mutation on 1st or 2nd line EGFR TKIs, Osimertinib is the treatment of choice
- For those with negative T790M mutation and symptomatic progression, platinum based chemotherapy is the standard therapy
- But for those with asymptomatic oligometastatic ( ≤ 3 sites ) progression on EGFR TKIs and with no T790M mutation, TKIs can be continued beyond progression along with local ablative therapies to delay platinum based chemotherapy
- For isolated CNS progression, continuing TKIs with local CNS ablative therapy is the standard of care, consider osimertinib only if local therapy is not possible and T790M mutation is positive

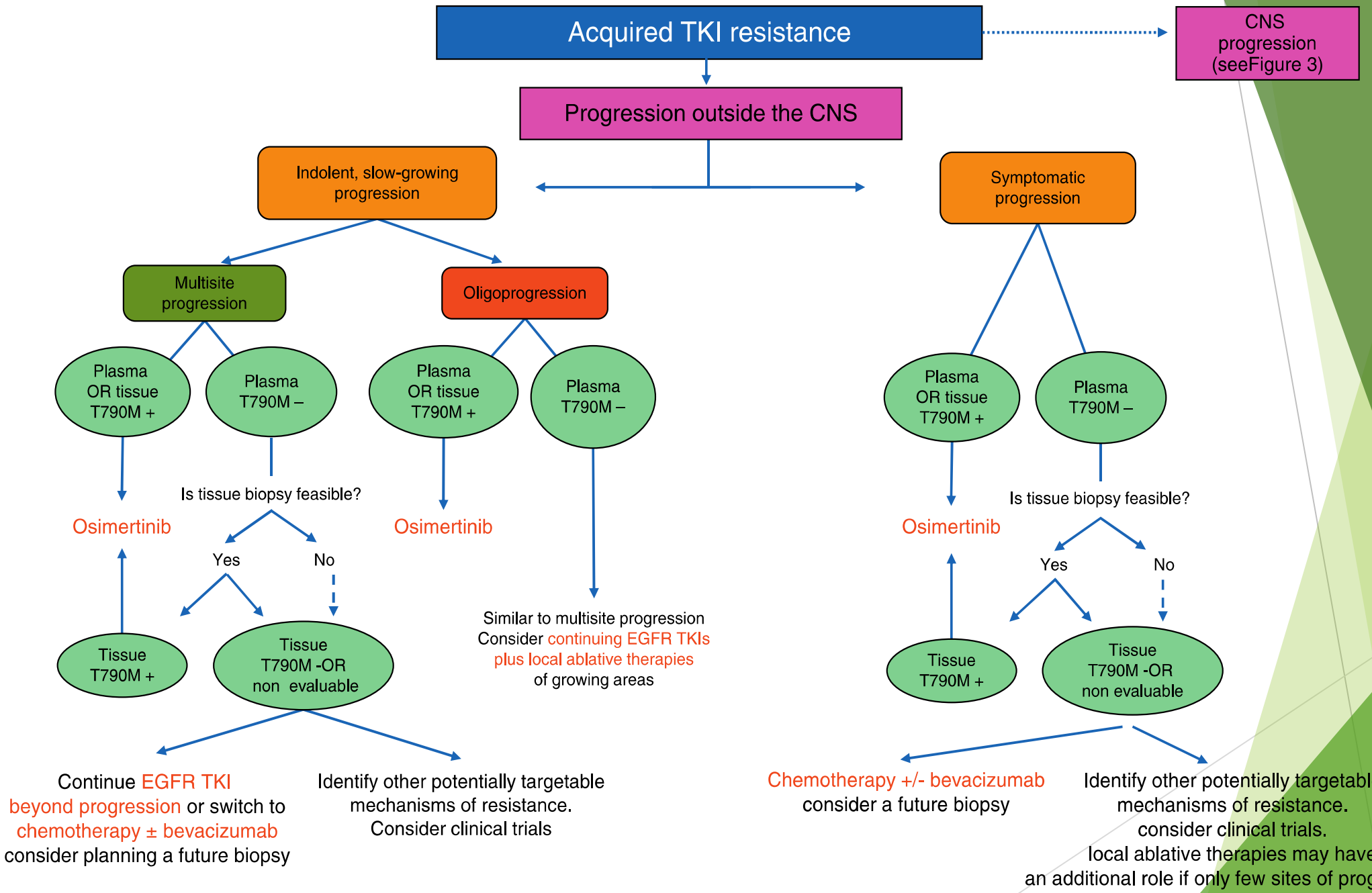
**Westover et al. *Annals of Oncology.* January 2018;29(1): i10–i19**

| STUDY | AURA phasel/II ( single arm prospective study) |
|---|---|
| SUBJECTS | EGFR-TKI–pretreated *EGFR*m- and T790M-positive advanced non–small-cell lung cancer (NSCLC) (n=201) |
| METHOD | Once-daily osimertinib 80 mg |
| RESULTS | ORR - 62% (95% CI, 54% to 68%)<br>Disease control rate - 90% (95% CI, 85 to 94).<br>Median duration of response - 15.2 months<br>Median PFS was 12.3 months (95% CI, 9.5 to 13.8)<br>Adverse events<br>Diarrhea - 43% ( ≤ 1 % grade 3 and above )<br>Rash – 40 % ( ≤ 1 % grade 3 and above )<br>Interstitial lung disease 4% |
| COMMENTS | EGFR approved for *EGFR*m T790M advanced NSCLC who progress after EGFR-TKI treatment<br>**BUT NO CONTROL USED** |

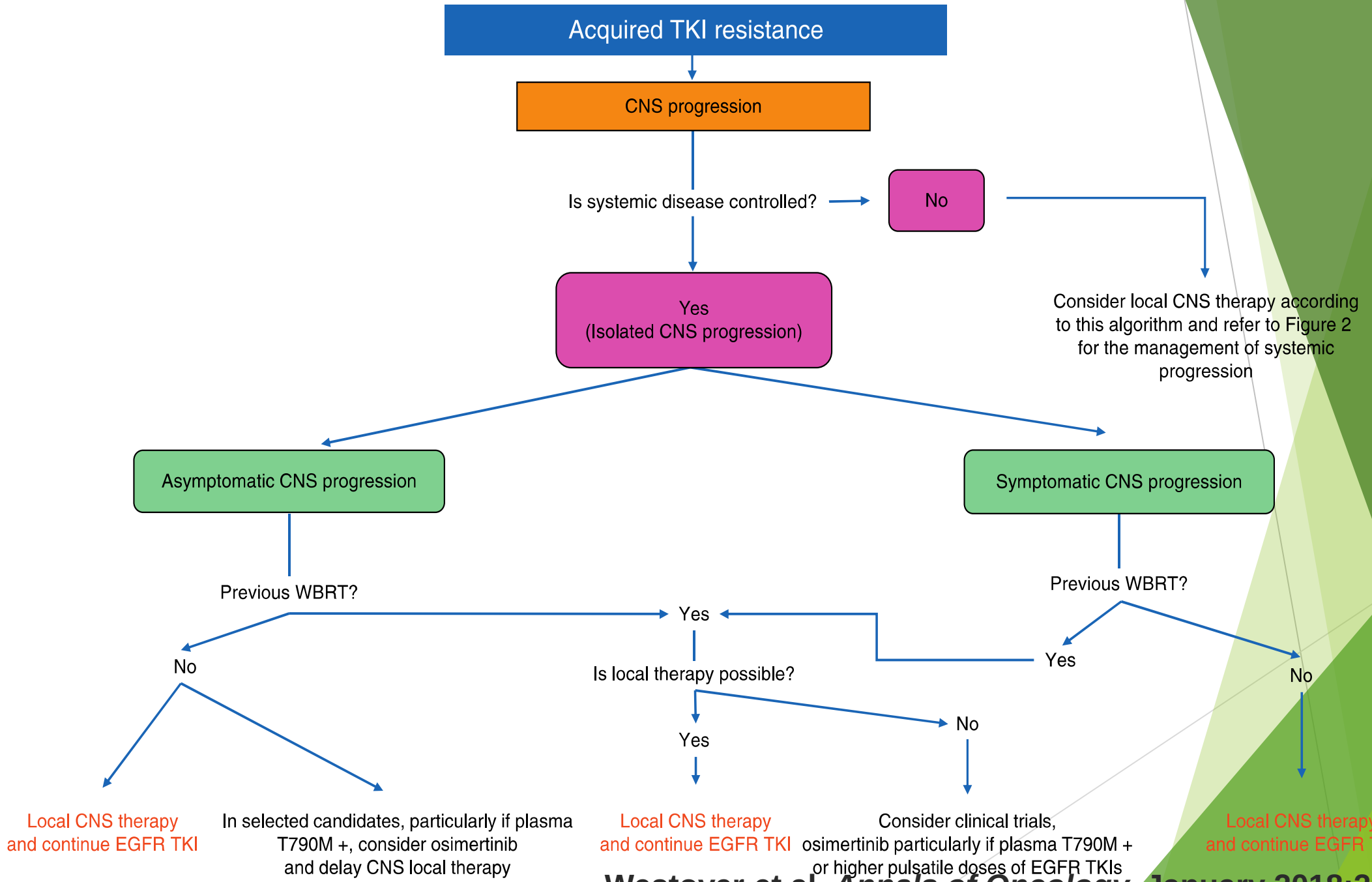
**James et al. Journal of Clinical Oncology 2017 35:12, 1288-12**

| STUDY | AURA phase 3( RCT) |
|---|---|
| SUBJECTS | EGFR-TKI–pretreated *EGFR*m- and T790M-positive advanced non–small-cell lung cancer ( n= 419) |
| METHOD | 2:1 ratio to either **oral osimetinib 80 mg** or i.v **pemetrexed** (500 mg per m2 BSA) plus either **carboplatin** (target AUC5) or **cisplatin** (75 mg per m2 ) every 3 weeks for up to six cycles; maintenance pemetrexed was allowed. |
| RESULTS | **PFS** 10.1 vs 4.4 ; HR 0.30<br>**ORR** 71 % (95% CI, 65 to 76)vs 31 % (95% CI, 24 to 40)<br>**Those with CNS metastases (n= 144) PFS** 8.5 vs 4.2<br>**Adverse Events** grade ≥3 -23 % vs 47% |
| COMMENT | Osimertinib had significantly **greater efficacy** than platinum therapy plus pemetrexed in patients with **T790M +ve advanced NSCLC (including those with CNS metastases) after disease progression** during first-line EGFR-TKI therapy. |

**Mok et al. N Engl J Med 2017; 376:629-640**




Westover et al. *Annals of Oncology.* January 2018;29(1): i10–i19




Westover et al. *Annals of Oncology*. January 2018;29(1): i10–i19

| STUDY | PROSPECTIVE STUDY by Ramalingam et al | FLAURA TRIAL( RCT) |
|---|---|---|
| SUBJECT | Treatment naïve Locally advanced or metastatic EGFR mutation +ve NSCLC patients (n=60) | Previously untreated, *EGFR* mutation-positive (exon 19 deletion or L858R) advanced NSCLC (n= 556) |
| METHOD | Osimertinib as first line 80 mg vs 160 mg | **1:1 ratio** osimertinib 80 mg OD vs gefitinib 250 mg OD/Erlotinib 150 mg OD |
| RESULTS | ORR 67% VS 87%<br>PFS **19.3 months**<br>Dose reduction for adverse effect done in 10% patients<br>MC adverse event - **diarrhoea** | PFS ( months) 18.9 vs 10.2<br>ORR 80 % vs 76%<br>DOR (months)17.2 vs 8.5<br>Survival at 18 months 83% vs 71%<br>Adverse events grade ≤ 3 34% vs 45% |
| COMMENT | Osimertinib is more effective than 1st generation TKIs as a first line therapy in EGFR mutant advanced NSCLC<br>This might be due to the **presence of minor subpopulation of T790M mutation +ve cells before starting TKIs** | |

Osimertinib approved as 1st line for EGFRm +ve in April 2018

**Soria et al. N Engl J Med 2018; 378:113-125**

**Ramalingam et al. J Thorac Oncol 2016 Apr;11(4 Suppl):S152**

# Other 3rd Gen EGFR TKI
# Rociletinib- not approved yet

| STUDY | PROSPECTIVE STUDY |
|---|---|
| SUBJECT | *EGFR*-mutated NSCLC on EGFR TKIs who had disease progression with/without T790M mutation<br>n=130 |
| METHOD | First 57 received Free from Rociletinib-150 mg OD to 900 mg BD<br><br>Next 73- Hydrogen Bromide form 500 mg BD to 1000mg BD |
| RESULT | Objective responses were consistently observed at a dose of **900 mg twice daily of the free-base form and all doses of the HBr form**<br>**RR** in T790M-positive tumors was **59%** (95% confidence interval [CI], 45 to 73), **29 % in T790M -ve patients**<br>**Disease-control rate** (the proportion of patients with a complete or partial response or stable disease) was **93%** (43 of 46 patients)<br>PFS - **13.1 months** (95% CI, 5.4 to 13.1)<br>Dose limiting adverse effect- Hyperglycemia -22% |
| COMMENT | Rociletinib is an alternative to Osimertinib but not approved yet and awaiting results on ongoing trials **Sequist et al. N Engl J Med. 2015 Aug 6;373(6):578-9** |

# TACKLING EMT- EPITHELIAL TO MESENCHYMAL TRANSFORMATION

- Intratumoral heterogeneity is a great hurdle
- **Combined inhibition of EGFR and the TGFß receptor** will prevent EMT
- Drug holiday could lower TGFß and thereby reestablish TKI sensitivity
- However TGFß inhibition cannot reverse EMT and **can only be used as a preventive measure**
- Histone deacetylase inhibitors have been shown to overcome EGFR TKI resistance linked to epigenetic changes and EMT state but with limited data

Suda et al. *Journal of thoracic oncology.* 2017;12(1):27-35
Soucheray. *Cancer research*. 2015;75(20):4372-4383.

# SMALL CELL TRANSFORMATION

- Most cases of SCLC transformation exhibit neuroendocrine differentiation with increased chemosensitivity
- They respond well to initial etoposide and cisplatin (EP) chemotherapy
- The response rate of the standard regime (cisplatin or carboplatin plus etoposide) was reported to be 70% to 90% for limited-stage disease and 60% to 70% for extensive stage disease
- Overall survival was reported to be **14 to 20 months and 9 to 11 months, respectively.**

Jiang et al. Xie. M, ed. *Medicine*. 2016;95(6):e2752.

# HER2 OVEREXPRESSION

| study | Preclinical study |
|---|---|
| SUBJECTS | NSCLC cell lines with known EGFR, K-ras, or ERBB2 mutations |
| METHOD | Pan ERBB Inhibitor - PF00299804<br>( later named as Dacomitinib ) and Geftinib on individual cell lines |
| RESULTS | KRAS mutants- both drugs ineffective<br>T790M mutants- Dacomitinib effective<br>ERBB2 mutations- Dacomitinib effective |
| COMMENTS | 2nd gen EGFR TKIs can be used to overcome acquired resistance to 1st gen TKIs via HER 2 overexpression |

Engelman et al. Cancer Res December 15 2007 ;67(24): 11924-11932

# TRIALS ON OVERCOMING EGFR RESISTANCE

| Resistant mechanism | Strategy | Clinical research |
|---|---|---|
| ***EGFR* mutation** | | |
| T790M | EGFR-TKIs combined/+antibodies | Afatinib+cexitumab |
| | T790M-specific inhibitors | CO-1686/AZD9291 |
| | c-Met inhibitors+PI3K inhibitors | GDC0973+GDC0941 |
| | HSP90 inhibitors | Luteolin/ganetespib |
| | EGFR-TKIs+MEK inhibitors | Afatinib+ARQ 197 |
| | Glycolysis inhibition+EGFR-TKIs | Afatinib+AUY922 |
| **Bypass pathway** | | |
| HER family abnormality | HER inhibitors+EGFR-TKIs | Afatinib/dacomitinib |
| *c-Met* amplification | EGFR-TKIs+c-Met inhibitors | Erlotinib+crizotinib |
| | | Dacomitinib+crizotinib |
| HGF overexpression | EGFR-TKIs+PI3K inhibitors | Gefitinib+PI-103 |
| | Triple inhibition of EGFR/Met/VEGF | – |
| IGFR abnormality | IGFR inhibitors+EGFR-TKIs | AG1024+gefitinib |
| EGFRvIII | EGFRvIII antibodies | – |
| VEGF/VEGFR abnormality | EGFR-TKIs+VEGF inhibitors | ZD6474 |
| | MEK inhibitors+VEGF inhibitors | ZD6474+PD0325901 |
| PDGF/PDGFR abnormality | EGFR-TKIs+PDGF inhibitors | – |
| FGF/FGFR abnormality | EGFR-TKIs+FGF inhibitors | – |
| IL-6 abnormality | IL-6 antibodies | Siltuximab |
| AXL abnormality | AXL inhibitors | NPS-1034 |
| *CRKL* amplification | Unknown | Unknown |
| Integrin beta1 overexpression | Unknown | Unknown |
| **Downstream pathway** | | |
| *K-RAS* mutations | PI3K inhibitors+MEK inhibitors | GDC-0941+AZD6244 |
| *BRAF* mutations | BRAF inhibitors+MEK inhibitors | Dabrafenib+trametinib |
| Loss of *PTEN* | mTOR inhibitors/AKT inhibitors | – |
| *PIK3CA* mutation | EGFR-TKIs+PI3K inhibitors | Gefitinib+BKM120 |
| Low expression of NF1 | Unknown | Unknown |
| **Apoptosis pathway** | | |
| *BIM* BH3 deletion | EGFR-TKIs+PP2A activator | Erlotinib+FTY720 gefitinib+FTY720 |
| **Histologic transformation** | | |
| EMT | EGFR-TKIs+MEK1/2 inhibitors | – |
| SCLC transformation | Platinum+VP16/EGFR-TKIs | – |
| **ABC effusion** | EGFR-TKIs+HER-2 inhibitors | GW583340/GW2974 |
| **Unknown mechanism** | EGFR-TKIs combined | Afatinib+cexitumab |
| | EGFR-TKIs+glycolysis inhibitors | Erlotinib+AUY922 |

# CNS SANCTUARY

- <2% of plasma drug concentration at steady state is detected in cerebrospinal fluids
- Concentration needed to inhibit EGFR above the IC50 is only maintained for a short time in the CNS at conventional dose
- Escalating doses of EGFR TKIs have been studied to increase CNS penetration
- **Previous studies suggested that 2nd gen Afatinib is effective on EGFR mutated advanced NSCLC patients with brain metastasis who have previously received 1st gen TKIs/ chemotherapy**
- With the **recent approval of Osimertinib as the 1st line therapy for EGFR mutated NSCLC, it has now become the treatment of choice for those with CNS involvement**

Kelly et al. *Frontiers in Oncology*. 2018;8:208..

Westover et al. *Annals of Oncology.* January 2018;29(1): i10–i19

# 2nd vs 1st Generation for CNS Mets

| STUDY | Prospective single arm trial |
|---|---|
| SUBJECT | NSCLC patients progressing after at least one line of chemotherapy and one line of EGFR-TKI treatment ( n=573) 100 patients had Brain metastasis |
| METHOD | Afatinib |
| RESULTS | Median **TTF for patients with CNS metastasis was 3.6 months, and did not differ from a matched group of 100 patients without CNS metastasis** |
| COMMENT | Afatinib might be used in those who have CNS progression after 1ST GEN TKI<br>**But recent studies including LUX LUNG 7 have defied this and suggest no significant difference in PFS between 1st and 2nd generation in BM patients** |

Hoffknecht et al. J Thorac Oncol .2015 Jan;10(1):156-63

# DOSE ESCALATION FOR CNS ??

| STUDY | Prospective open label single centre |
|---|---|
| SUBJECTS | EGFR mutated Advanced NSCLC n= 34 ( 11/34) had BM |
| METHOD | Twice weekly pulsatile Erlotinib plus daily oral Erlotinib 50 mg |
| RESULT | MTD- erlotinib 1200 mg days 1–2 and 50 mg days 3–7 weekly.<br>Median PFS- 9.9 months (95% CI 5.8–15.4 months).<br>No patient had progression of an untreated CNS metastasis or developed a new CNS lesion while on study (0%, 95% CI 0–13%).<br>The most frequent toxicities (any grade) were rash, diarrhea, nausea, fatigue, and mucositis ( **no significant increase** ) |
| COMMENT | This dosing schedule **prevented progression of untreated or any new central nervous system metastases in all patients**<br>**No improvement in PFS**<br>But only 11 (very few patients had CNS involvement at baseline) |

Yu et al. Ann Oncol. 2017 Feb 1;28(2):278-284

| Study | Phase | Tyrosine kinase inhibitors therapy | EGFR mutant NSCLC patients with BM (unless specified) | Response rate (%) | Survival (months) |
|---|---|---|---|---|---|
| Park 2012 | II | Erlotinib or gefitinib | 28 | Partial Response (PR): 83<br>Stable Disease (SD): 11 | Progression-free survival (PFS): 6.6<br>Overall Survival (OS): 15.9 |
| Yu 2017 | I | Pulsatile erlotinib | 34 (only 32% had brain mets) | Complete Response (CR): 2<br>PR: 70 | PFS: 9.9 |
| Iuchi 2013 | II | Gefitinib | 41 | Objective response rate (ORR): 88 | PFS: 14.5<br>OS: 21.9 |
| Yang 2017 (BRAIN) | III | Icotinib | 85 | – | Intracranial PFS: 10.0 |
| Schuler 2016 (LUX-Lung 3/6) | III | Afatinib | 25/46 | – | PFS: 11.1/8.2 |
| Park 2016 (LUX-Lung 7) | II | Afatinib | 26 | – | 8.4 |
| Mok 2017 (AURA 3) | II | Osimertinib | 144 (T790M mut) | – | PFS: 8.5 |
| Goss 2017 (AURA/AURA2) | II | Osimertinib | 50 (T790M mut) | Central nervous system (CNS) ORR: 54 | – |
| Yang 2017 (BLOOM) | I | Osimertinib | 32 (LM, 11 T790M mut) | ORR: 43 | – |
| Soria 2017 (FLAURA) | III | Osimertinib | 53 | ORR: 75<br>CNS PD: 6 | PFS: 15.2 |

Kelly et al. *Frontiers in Oncology*. 2018;8:208..

# HSP90 INHIBITION- DOES IT WORK?

| STUDY | PRECLINICAL STUDY |
|---|---|
| SUBJECTS | xenograft NSCLC tumor cells with and without T790M mutation |
| METHOD | Erlotinib + HSP90 inhibitor ganetespib vs Erlotinib alone in cells with and without T790M mutation |
| RESULTS | Combination therapy improved tumour regression in T790M –ve cells<br>Significantly improved tumor growth inhibition in T790M +ve cells |
| Comment | Human trials pending to validate TKI+ **Ganetespib** to tackle T790M mutation |

Hsp 90 ( Heat Shock protein) is a chaperone protein that stabilizes T790M mutated EGFR

Smith et al. *Targeted Oncology*. 2015;10(2):235-245.

# USE OF COLA DRINKS

| STUDY | Randomised cross over study |
|---|---|
| SUBJECTS | NSCLC patients n=28 |
| METHOD | Intrapatient differences in absorption after a 7-day period of concomitant treatment with erlotinib, with or without esomeprazole, with either cola or water |
| RESULTS | Patients treated with **erlotinib and esomeprazole with cola,** the mean AUC0-12h **increased 39%** (range, -12% to 136%; P = .004), whereas in **patients not treated with the PPI, the mean AUC0-12h was only slightly higher** (9%; range, -10% to +30%; P = .03) after erlotinib intake with cola. |
| COMMENT | **Cola increased the bioavailability of erlotinib in those taking PPIs**<br>**However only marginal benefit in those not taking PPIs** |

Leeuwen et al. J Clin Oncol. 2016 Apr 20;34(12):1309-14

# EGFR TKI PLUS TS-1(THYMIDYLATE SYNTHASE)- NOVEL COMBINATION THERAPY

- Oral agent
- A preclinical study illustrated that **gefitinib could decrease the expression of the thymidylate synthase (TS)**, an assumed mechanistic driver of TS-1 resistance in lung cancer cells.
- TS-1 is also reported to have a **synergistic antiproliferative effect with gefitinib** in male athymic nude mice, **regardless of T790M status and MET amplification**

Yang et al. *Thoracic Cancer*. 2018;9(6):693-698.

# EGFR TKI + TS-1

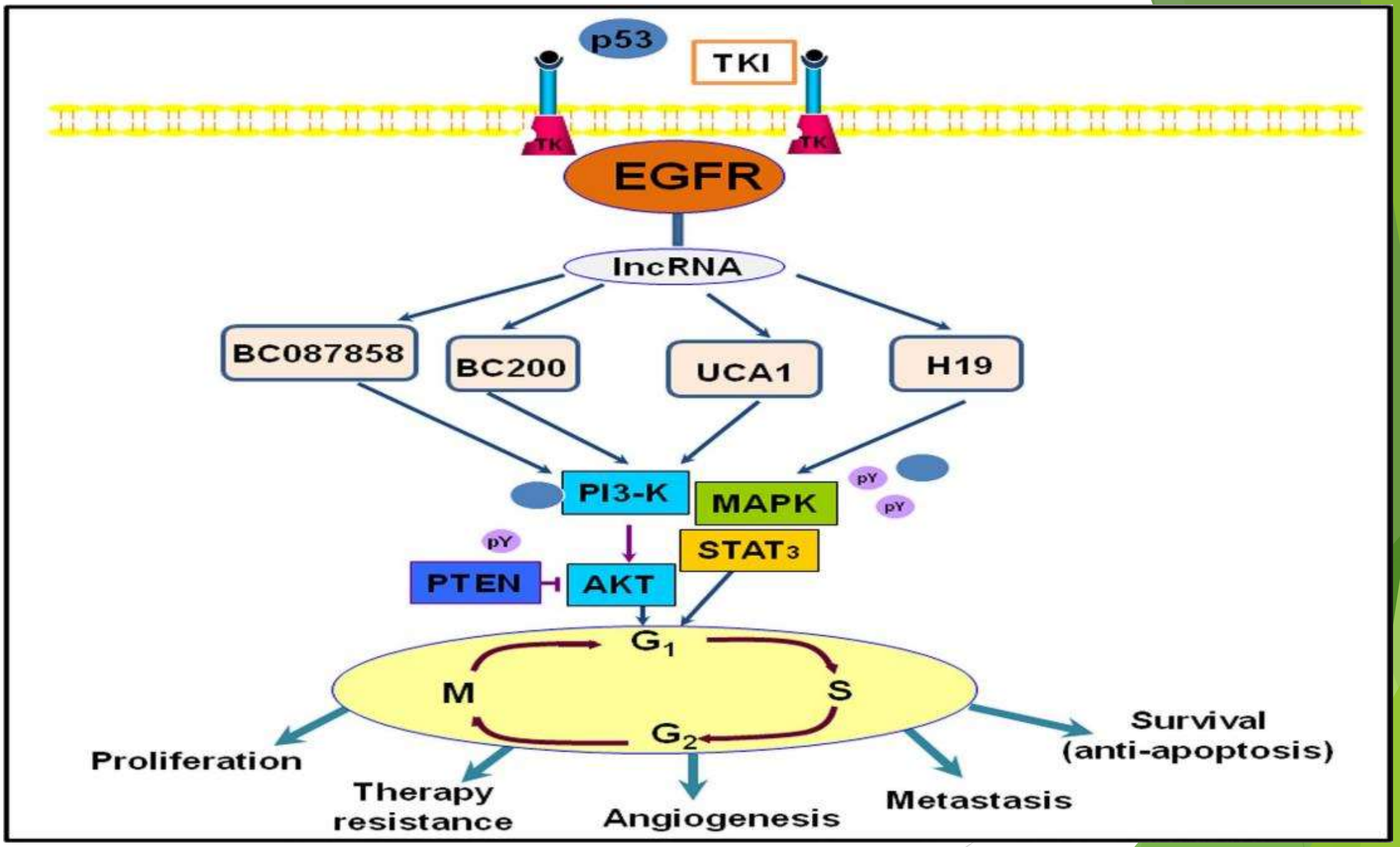
| STUDY | Phase II, single-arm and single-center prospective study. |
|---|---|
| SUBJECT | Stage IIIB–IV NSCLC patients with acquired resistance to prior EGFR-TKI treatment ( n= 42) |
| METHOD/INTERVENTION | EGFR TKI + TS-1 |
| RESULTS | OS - 31.9 (95% CI 17.8–46.0) months<br>DCR- 69.0% (29/42)<br>No grade 4 toxicity or grade 3 hematologic toxicity |
| COMMENTS | Needs to be validated by larger prospective clinical trials. |

Yang et al. *Thoracic Cancer*. 2018;9(6):693-698.

# LATEST DEVELOPMENTS-LncRNA

- Long non-coding RNAs (lncRNAs) are non-coding RNAs
- involved in a number of biological processes
- lncRNA UCA1 may stimulate non-T790M acquired resistance for EGFR-TKIs by activating the AKT/mTOR pathway and EMT
- Future target for assessing non T790M mediated EGFR resistance and therapy

Liu et al. Oncotarget. 2017 Jul 25;8(30):50209-50220.




Liu et al. Oncotarget. 2017 Jul 25;8(30):50209-50220

# LATEST DEVELOPMENTS- microRNAs

- Small noncoding RNAs that act as key post-transcriptional regulators of gene expression.
- miRNAs can be used as a predictive biomarker of response to EGFR TKIs
- number of miRNAs, such as miR-200a, miR- 27a/27b, miR-133a, and miR-134
- miRNA-based therapy could possibly be utilized to target EGFR
- miR- 34a is considered to be the most likely of the miRNAs to become a diagnostic marker and target of drug therapy in NSCLC

Liu et al. Oncotarget. 2017 Jul 25;8(30):50209-50220.

# OSIMERTINIB RESISTANCE

1. EGFR dependent mechanisms
2. EGFR Independent mechanisms

**Z.-H. Tang et al. Cancer Letters (2018), doi: 10.1016/j.canlet.2018.02.004**.

# EGFR DEPENDENT OSIMERTINIB RESISTANCE

- **C797S mutation-** mutation of the binding site of osimertinib on EGFR affecting its covalent binding
- Other EGFR mutations G796D, G796S/R, L792F/Y/H, C797S/G - decreased the binding activity of OSI to EGFR
- Amplification of *EGFR* ex19del and wild type and loss of *EGFR* T790M
- Low expression of EGFR protein

Z.-H. Tang et al. Cancer Letters (2018), doi: 10.1016/j.canlet.2018.02.004.

# EGFR INDEPENDENT OSIMERTINIB RESISTANCE

- Overexpression of **HER2 or MET amplification**
- Overexpression of **Fibroblast growth factor receptor 1 (FGFR1) and basic fibroblast growth factor (FGF2)**
- Increased levels of *NRAS* E63K and Q61K mutation, *KRAS* G12S mutation, *NRAS* copy number, or *KRAS* copy number
- Mutation of ***BRAF* V600E**, ***PIK3C*** E545K, ***PTEN*** Y27C, *CTNNB1* S37F, and *TSC2* N486I; deletion of *PTEN*; and overexpression of MAPK1, AKT3, and AXL
- **Small cell transformation**
- **EMT**
- **Increased PDL1 expression**

Z.-H. Tang et al. Cancer Letters (2018), doi: 10.1016/j.canlet.2018.02.004.

# TREATING EGFR DEPENDENT OSIMERTINIB RESISTANCE
# NOTE: NO DRUG APPROVED TILL DATE

**C797S MUTATION**

- **EGFR TKIs**
  - If **C797S and T790M mutations occur in cis**, then the patients are **resistant to first-, second-, and third-generation EGFR TKIs**
  - If **C797S and T790M mutations occur in trans**, then the **combination of first- and third-generation EGFR TKIs** might be the therapeutic strategy for patients
- **Monoclonal Antibodies**

  Panitumumab or cetuximab, alone or in combination with other drugs
- **Brigatinib ( ALK + EGFR inhibitor) ± anti EGFR antibody**

  In mice models

Z.-H. Tang et al. Cancer Letters (2018), doi: 10.1016/j.canlet.2018.02.004.

# TREATING EGFR INDEPENDENT OSIMERTINIB RESISTANCE

- **OVEREXPRESSION OF HER2 -** Combination treatment of OSI and MEK inhibitor
- **MET AMPLIFICATION-** crizotinib alone or in combination with OSI
- **SCLC TRANSFORMATION-** platinum-based doublet chemotherapy ( paclitaxel favoured)
- **PDL1 EXPRESSION > 50 %**- Pembrolizumab can be used

  (also advised in NCCN guidelines for this indication)

Z.-H. Tang et al. Cancer Letters (2018), doi: 10.1016/j.canlet.2018.02.004.

# TAKE HOME MESSAGE

- Exon 19 deletion and L858R mutations are the most common TKI sensitising mutations
- Earlier, 1st and 2nd generation TKIs ( Geftinib, Erlotinib and Afatinib ) used to be the drugs of choice for 1st line therapy in advanced NSCLC with these activating mutations
- However almost all these patients progress after a median period of 12 months
- Ensure proper dosing and avoid drug interactions before diagnosing disease progression
- T790M mutation is the most common cause of acquired resistance to TKIs
- Recent studies indicate that subclones of tumour cells possess these mutations even before TKI therapy giving a survival advantage for these subclones

# TAKE HOME MESSAGE

- Osimertinib is the cornerstone to treat these patients
- But methods to manage other less common causes of primary and secondary resistance to EGFR TKIs are being studied
- It is imperative  that future studies be conducted on NSCLC patients to diagnose even rarer mutations and to tackle them to improve survival outcomes